AD_____

Award Number: W81XWH- 08-1-0529

TITLE: Genome Wide Association Study to Identify SNPs and CNPs Associated with Development of Radiation Injury in Prostate Cancer Patients Treated with Radiotherapy

PRINCIPAL INVESTIGATOR: Barry S. Rosenstein, Ph.D.

CONTRACTING: Mount Sinai School of Medicine
New York, NY 10029-6574.

REPORT DATE: October 2012

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
15 October 2012	Final	30 September 2008 – 29 September 2012

4. TITLE AND SUBTITLE

Genome Wide Association Study to Identify SNPs and CNPs Associated with Development of Radiation Injury in Prostate Cancer Patients Treated with Radiotherapy

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-08-1-0529

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Harry Ostrer, M.D. and Barry S. Rosenstein, Ph.D.

E-Mail: barry.rosenstein@mssm.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Mount Sinai School of Medicine
New York, NY 10029

NYU School of Medicine
New York, NY 10016

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
The hypothesis that forms the basis for this research is that patients who possess certain SNPs or CNPs are at a greater risk for developing severe urinary morbidity or erectile dysfunction (ED) resulting from radiotherapy for prostate cancer. The specific aim of this project was to identify through a genome wide association study the SNPs and CNPs associated with the development of these adverse effects resulting from the use of radiation to treat prostate cancer. We have performed a two-stage genome-wide association study and identified and validated loci associated with each of these primary outcomes. It is expected that the results of this work will provide the basis for a clinically relevant predictive test to identify patients at increased risk for development of adverse events following radiotherapy. It is anticipated that such a tool will be used to aid clinicians in personalizing treatment to improve the therapeutic outcome for men diagnosed with prostate cancer.

15. SUBJECT TERMS
Radiation, SNP and CNP genotyping, normal tissue toxicities

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	47	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

INTRODUCTION:

Radiotherapy can provide a sustainable cure for prostate cancer and has become accepted as a standard treatment option. However, some men develop side effects following treatment, including urinary morbidity and erectile dysfunction, which have a substantial effect on quality of life. These side effects vary in duration and severity, and while most patients return to baseline symptom levels after a year, a subset of patients experience more severe and lasting effects. A predictive assay that could identify such patients could be used to help tailor treatment plans. Previous research on radiation induced injury in breast cancer patients suggests that the variation in such side effects is largely due to patient-specific, possibly genetic effects rather than treatment differences or random effects. The purpose of this study is to identify genetic polymorphisms associated with development of urinary morbidity or erectile dysfunction following radiotherapy for prostate cancer. The medical application of these findings will be to develop a risk assessment genetic test to assist physicians and patients in making informed decisions on the course of therapy for prostate cancer. Physicians and patients could together weigh the benefits of therapy with the individualized risk of developing radiation side effects and could then customize the treatment course.

BODY:

Year 1: The main efforts during the first year of the project were an intensive review of the clinical data for each subject in this study to verify their inclusion in this study, and piloting of laboratory methods. Thus, efforts were focused on the following tasks: patient follow-up, finalization of inclusion criteria, case definitions and preparation of high quality genomic DNA for microarray analysis. We completed patient follow-up pertaining to urinary outcomes (International Prostate Symptom Score, IPSS) and erectile dysfunction (Sexual Health Inventory for Men score and Mount Sinai Erectile Function Score) for the minimum time period for all individuals in our database. Case and control definitions were modified based on clinical characteristics of our patient set and findings in recently published reports.

Our database now includes over 3,000 men treated with brachytherapy and followed-up for a minimum of one year. We collected blood samples and prepared genomic DNA from 858 patients who me the inclusion criteria for the study of having at least one year of follow-up and baseline symptom assessment for one or more of our primary outcomes. The included patients were followed with assessment of urinary outcomes using the IPSS questionnaire and erectile dysfunction using the SHIM and MSEF questionnaires as planned. Demographic and clinical data for the 858 patients for whom we have DNA samples were analyzed to confirm that cases and controls were similar with respect to potential confounders (Table 1).

The patients included in the study were selected based on the criteria and case definitions outlined in the initial proposal with minor modifications based on clinical characteristics of the patients in our database and recently published findings regarding radiation injury outcomes. First, we decreased the minimum follow-up time for inclusion in the study from two years to one year. Our data as well as recent reports tracking the same radiotherapy adverse effects suggested that common acute urinary and sexual dysfunction effects take place within the first 12 months post-treatment (Keyes, 2009; Aaltomaa, 2009; Tanaka, 2009). Twelve months appeared to be sufficient time to separate out those individuals who experience long-term symptoms that may have a genetic basis. We included patients in the study who were treated with either I-125 seed implant alone or in combination with external beam radiation therapy. There is no constant evidence in the literature to suggest that the effects on urinary or erectile function are different in the monotherapy versus the combination therapy (Lee, 2006; Hurwitz, 2008). Dosimetric measurements were collected for each patient and only patients whose dose to the prostate (D90) was within the range of 160-180 Gy were included regardless of treatment type.

We removed the constraint on ethnicity for inclusion in the study as requested by the DOD Human Research Protection Office (HRPO). We had initially restricted inclusion to white, non-Hispanic patients in an effort to reduce identification of false positive associations due to population stratification. However, we were able to include a multi-ethnic patient population and control population stratification by performing principal components analysis and adjusting for principal components in association tests.

We had initially planned to analyze urinary morbidity as a case-control outcome, but analysis of the distribution of IPSS scores showed that it would be more appropriate to treat this outcome as a quantitative trait. Thus, we treated the change in IPSS relative to pre-treatment as a continuous outcome measure of radiation-induced urinary morbidity, adjusting for pre-treatment score in genetic association tests. This definition allows for inclusion of individuals who report a less severe long-term response but, relative to their pre-treatment status, still experience a substantial decline in urinary

symptoms. This case definition better accounts for the subjective nature of the IPSS test, the normal distribution of IPS scores, and the variability in long-term urinary morbidity from moderate to severe.

With regard to erectile dysfunction, we had initially planned to exclude from the study patients who have taken phosphodiesterase inhibitors (PDIs) to treat erectile dysfunction as that may itself be associated causally with the outcome. Upon closer examination of the data we found that a substantial percentage of patients reported using PDIs, and if we included patients who reported using PDIs, there was only a small difference in usage between cases and controls. Rather than exclude these patients and reduce our sample size, we included these patients and control for PDI usage in the test for association.

During the first year of the project we ran a pilot set of 5 Affymetrix 6.0 microarrays to confirm the quality of our DNA samples and check the protocol for the arrays. We achieved over 99% call rates with these 5 pilot samples. We had previously run 83 Affymetrix 6.0 arrays on a separate patient set and were able to use the quality control results from this set to make adjustments to our protocol, resulting in the high DNA quality and genotyping call rates for the pilot samples from the current study.

Table 1. Demographic and clinical characteristics of patients included in the discovery and validation GWAS of urinary morbidity and erectile dysfunction following prostate radiation therapy.

	Urinary Morbidity	**Erectile Dysfunction**	
	N = 723	**Cases N = 260**	**Controls N = 205**
Age*, mean (sd)	64.4 (7.7)	66.5 (6.7)	60.5 (6.9)
Race, N(%)			
Caucasian	551 (76.2%)	197 (75.8%)	161 (78.5%)
African American	90 (12.4%)	27 (10.4%)	25 (12.2%)
Hispanic	53 (7.3%)	25 (9.6%)	10 (4.9%)
Asian	12 (1.7%)	6 (2.3%)	2 (1.0%)
Not known	4 (0.6%)	5 (1.9%)	7 (3.4%)
Initial PSA, mean (sd)	8.1 (7.8)	10.3 (20.6)	7.1 (5.2)
Stage, N (%)			
T1	370 (51.2%)	119 (45.8%)	130 (63.4%)
T2	328 (45.4%)	131 (50.4%)	70 (34.1%)
T3	25 (3.5%)	10 (3.8%)	5 (2.4%)
Gleason Score, N(%)			
≤6	442 (61.1%)	141 (54.2%)	146 (71.2%)
7	200 (27.7%)	76 (29.2%)	47 (22.9%)
≥ 8	81 (11.2%)	43 (16.5%)	12 (5.9%)
Treatment Type, N(%)			
Implant Only	406 (56.2%)	114 (43.8%)	128 (62.4%)
Implant + EBRT	317 (43.8%)	139 (53.5%)	72 (35.1%)
EBRT Only	0	7 (2.7%)	5 (2.4%)
Mean length of follow-up (months)	56.0	64.7	54.3

Year 2: Efforts in the second year of funding were focused on completion of the discovery phase of the genome-wide association study. Genomic DNA from the 386 prostate cancer patients randomly selected for the Discovery Cohort was assayed using Affymetrix SNP6.0 arrays.

A considerable amount of effort was spent on quality control checks to ensure sample identity and to assess and minimize risks of batch effects and population stratification. The 386 samples were run in 5 batches (i.e. 5 96-well plates). We incorporated two types of controls for each batch: an external control set comprising a HapMap trio (two parents and an offspring) and an internal control set comprising three duplicates of randomly selected prostate cancer patient samples. Initial overall genotyping rate among all 411 samples (study samples plus controls) was > 97%. We were able to confirm >99% reproducibility of genotype calls among the four batches by comparing the HapMap samples across batches. We also calculated identity-by-descent (IBD) and identity-by-state (IBS) measures to confirm the identity of the control samples and identify any patient samples with greater-than-expected similarity. We obtained expected IBD and IBS values for all controls: approximately 50% IBD sharing between the offspring and each parent of the HapMap trios, and >98% IBS sharing between identical pairs for all duplicate samples. Several prostate cancer samples were excluded based on greater-than-expected IBD sharing (8 pairs of samples) or low call rate (<90%; 2 samples). The final dataset contained 367 samples with call rate >98%.

Because the study involved a multi-ethnic patient population, the genetic population structure was assessed using principle components analysis and ancestry estimation using the program STRUCUTRE v2.1. As expected, based on self-reported race/ethnicity, approximately 78% of patients share ancestry primarily with Caucasian populations, approximately 4% share ancestry with Asian (Chinese and Japanese) populations, and approximately 18% are admixed with ancestry shared between African and Caucasian populations. For several patients with missing data on race/ethnicity, estimation of ancestry using SNP genotypes allowed us to accurately assign proportion shared ancestry and include those individuals in the analysis. After adjusting for the first five principal components in association tests, we obtained low genomic inflation factors of 1.02 for the ED patients and 1.00 for the urinary morbidity patients, suggesting population stratification was adequately controlled.

Association tests were carried out in the Discovery Cohort samples using logistic regression for ED and linear regression for urinary morbidity. We investigated four possible genetic inheritance models: allelic, genotypic, dominant and recessive. As outlined in our proposal, we set a fairly liberal cut-point of $p < 10^{-4}$ for inclusion in the validation study. This two-stage study design allowed us to capture most true positive associations and then filter out false positive associations through the validation study. In total, we identified 1,374 SNPs associated with urinary morbidity and 940 SNPs associated with ED that were investigated further in the validation study. We also identified 28 CNP sites that showed moderate association with either ED or urinary morbidity and were included in the validation study.

Years 3 and 4: Efforts in the third year of funding and the subsequent one-year no-cost extension were focused on the validation phase of the genome-wide association study, publication of results, and the follow-up fine-mapping study. For the validation phase, we genotyped and analyzed 493 patients comprising the Validation Cohort. We identified a set of SNPs that replicated in both cohorts, and then designed a high-density SNP array to perform fine-mapping with the aim of narrowing in on the specific region being tagged by the associated SNPs.

Advances in technology that took place during the second year of the project allowed us to increase both our SNP selection limit and sample size for the validation study. For similar cost to doing TaqMan assays as planned, we were able to build a custom microarray using Illumina's Infinium iSelect HD custom genotyping platform to genotype samples in the validation cohort. This allowed us to select approximately 1% of the SNPs from the discovery cohort for validation rather than the more modest numbers that would have been feasible using the TaqMan assay. Furthermore, for the same cost, we were also able to increase our sample size for the validation cohort from ~300 to 493 patients.

Because this study involves a multi-ethnic patient population, and ancestry was adjusted for in the analysis of the discovery phase data, we included approximately 1,000 ancestry-informative markers (AIMs) on the custom array being used in the validation study. To do this, we performed principle components analysis using reference populations from three sources: the International HapMap Project, the Population Reference Sample (POPRES), and the Human Genome Diversity Project (HGDP). We selected SNPs with minor allele frequency differences between pairs of reference populations, and then, using principle components analysis, tested the ability of various sized panels of selected 'ancestry-informative' SNPs to distinguish the ethnically and geographically distinct reference populations. We compared the performance of our ancestry-informative SNPs to a random selection of 100,000 SNPs which is typically used for principle components analysis and found that we could adequately stratify population groups using these AIMs.

Genotyping for the validation phase was completed successfully mid-way through the third year of funding. We achieved a high call rate and positive results from our control samples. Specifically, duplicate control samples included in both rounds of genotyping showed >95% concordance, and three control samples (a trio of two parents and an offspring) included on both the Affymetrix and Illumina genotyping platforms showed >99% concordance across platforms. We carried out the analyses for the validation phase of the GWAS and calculated combined p-values to identify SNPs significantly associated with each outcome.

For the ED outcome, 12 SNPs that were identified in the discovery cohort were validated in the replication cohort (Fisher combined p-values 2.1×10^{-5} to 6.2×10^{-4}). Interestingly, one of the top SNPs resides in an intron of the 17-beta-hydroxysteroid dehydrogenase II gene (*HSD17B2*), which catalyzes the oxidative metabolism of androgens and estrogens in human peripheral tissues, providing biological plausibility for association with ED. In a multivariable model including non-genetic risk factors, the odds ratios for the replicated SNPs ranged from 1.6 to 5.6 in the pooled cohort. We calculated an additive SNP score and found that there was a striking relationship between the cumulative number of SNP risk alleles an individual possessed and ED status (Sommers' D p-value = 1.7×10^{-29}). Specifically, a one-allele increase in cumulative SNP score increased the odds for developing ED by a factor of 2.2 (p-value = 2.1×10^{-19}). The cumulative SNP score model had a sensitivity of 84% and specificity of 75% for prediction of developing ED at the radiotherapy planning stage. The results of this part of the GWAS were published in the International Journal of Radiation Oncology, Biology and Physics. A copy of the published manuscript is included as an appendix.

For the urinary morbidity outcome, a region on chromosome 9p21.2 tagged by 8 SNPs showed the strongest association with change in IPSS in both the discovery and replication cohorts. Out of the group, SNP rs10967965 showed the strongest association signal with a beta coefficient of 2.7 (95% CI 1.2, 4.1) in the discovery cohort and 2.4 (95% CI 1.1, 3.6) in the replication cohort (discovery cohort p-value = 3.7×10^{-4}, replication cohort p-value = 1.9×10^{-4}; combined p-value = 6.6×10^{-7}) at the 2-3yr follow-up period. Individuals with the risk allele for rs10967965 experienced, on average, a 4.7

point increase in IPSS during the 2-3 year follow-up period whereas individuals without the risk allele for rs10967965 experienced, on average, a 2.6 point increase in IPSS during the 2-3 year follow-up period. For all 8 SNPs in this region, a positive association with change in IPSS is seen across all time periods, though the magnitude of effect and statistical significance is greatest during the 2-3 year time period. Additional support for this association is given by the finding that these SNPs reside in a haplotype block, which itself is associated with change in IPSS. In addition to the region on 9p21.2, we identified 24 SNPs tagging 10 genomic loci that showed moderate significance for association with change in IPSS at multiple follow-up periods across both the discovery and replication cohorts. We are in the process of submitting the results of this part of the GWAS to the Journal of Urology. A copy of the manuscript to be submitted is included as an appendix.

We sought to replicate the CNP associations for each outcome identified in the discovery phase of the study. However, upon analysis of the validation phase data, none of the CNPs appeared to be replicated. The association p-values from the discovery phase were quite modest for the identified CNP, so it was not surprising that none of them replicated. Given that CNPs are rarer and less well studied, a larger study with a more targeted design may be needed to better investigate whether copy number alterations are associated with normal tissue response to radiation therapy.

KEY RESEARCH ACCOMPLISHMENTS:

Year 1:

Refined and finalized inclusion criteria and case definitions for patients to be included in the study

Verified IPSS and SHIM/MSEF scores for a minimum of one year for all patients included in the study

Analyzed demographic and clinical characteristics of patients for whom we have blood collected to ensure similarity of cases and controls for each outcome

Established assays in our laboratory for the validation of the SNPs and CNPs that appear significantly associated with either urinary morbidity or erectile dysfunction in the initial training set and have successfully SNP and CNP genotyped patient samples.

Year 2:

Ran SNP/CNP genotyping arrays for 411 patient samples and controls in the discovery cohort

Achieved >98% call rate in final set of 367 patients after performing QC steps

Confirmed cases and controls were matched on race/ethnicity for all three outcomes and obtained ancestry estimates for each patient to include in logistic regression models for SNP association

Identified approximately 2,500 SNPs associated with ED or urinary morbidity to be investigated in the validation cohort

Completed patient recruitment to fulfill the sample size requirement for the validation study

Years 3 and 4:

Designed and built a mid-plex custom SNP microarray to genotype approximately 2,500 SNPs identified in the discovery phase of the project as well as approximately 1,000 ancestry-informative markers

Genotyped approximately 500 patients comprising the validation cohort for discovery phase SNPs and candidate SNPs

Completed the validation phase of the GWAS and identified 12 loci associated with ED and 11 loci (including on haplotype block tagged by 8 SNPs) associated with urinary morbidity.

Published the results of the ED outcome and completed a manuscript on the urinary morbidity outcome to be submitted for publication.

Presented results of the study in abstracts at the 54th annual meeting of the American Society of Therapeutic Radiation Oncology (ASTRO)

REPORTABLE OUTCOMES:

We have identified 157 SNPs associated with urinary morbidity (p-values $6x10^{-7}$ to 10^{-4}) and 167 SNPs associated with ED (p-values $2x10^{-7}$ to 10^{-4}). It is anticipated through validation in independent cohorts that a subset of these SNPs will form the basis of a robust clinical assay to predict which patients are most susceptible for the development of either urinary morbidity or ED following radiotherapy.

CONCLUSIONS:

We have performed a two-stage genome-wide association study to identify genetic variants associated with susceptibility for the development of either urinary morbidity or ED. We have identified and validated loci associated with each of these primary outcomes. The results of this work will provide the basis for a clinically relevant predictive test to identify patients at increased risk for development of adverse events following radiotherapy. It is anticipated that such a tool will be used to aid clinicians in personalizing treatment to improve the therapeutic outcome for men diagnosed with prostate cancer.

REFERENCES:

Aaltomaa SH, Kataja VV, Lahtinen T *et al.* Eight years experience of local prostate cancer treatment with permanent I125 seed brachytherapy--morbidity and outcome results. *Radiother Oncol.* 2009 May;91(2):213-6.

Cavalli-Sforza, L.L., *The Human Genome Diversity Project: past, present and future.* Nat Rev Genet, 2005. **6**(4): p. 333-40.

Hurwitz MD, Halabi S, Ou SS *et al.* Combination external beam radiation and brachytherapy boost with androgen suppression for treatment of intermediate-risk prostate cancer: an initial report of CALGB 99809. *Int J Radiat Oncol Biol Phys.* 2008 Nov 1;72(3):814-9.

International HapMap Consortium, *The International HapMap Project.* Nature, 2003. **426**(6968): p. 789-96.

Keyes M, Miller S, Moravan V, *et al.* Urinary symptom flare in 712 125I prostate brachytherapy patients: long-term follow-up. *Int J Radiat Oncol Biol Phys.* 2009 Nov 1;75(3):649-55.

Lee WR, DeSilvio M, Lawton C, *et al.* A phase II study of external beam radiotherapy combined with permanent source brachytherapy for intermediate-risk, clinically localized adenocarcinoma of the prostate: preliminary results of RTOG P-0019. *Int J Radiat Oncol Biol Phys.* 2006 Mar 1;64(3):804-9.

Nelson, M.R., et al., *The Population Reference Sample, POPRES: a resource for population, disease, and pharmacological genetics research.* Am J Hum Genet, 2008. **83**(3): p. 347-58.

Skol, A.D., et al., *Joint analysis is more efficient than replication-based analysis for two-stage genome-wide association studies.* Nat Genet, 2006. **38**(2): p. 209-13.

APPENDICES:

Kerns S, Stock R, Stone N, Ostrer H, Rosenstein B. *Genome Wide Association Study to Identify Genetic Variants Associated with Urinary Symptoms Following Radiotherapy for Prostate Cancer.* Poster presented at to the 54th annual ASTRO meeting (2012).

Buckstein M, Kerns S, Stock R, Stone N, Ostrer H, Rosenstein B. *Genome Wide Association Study to Identify Genetic Variants Associated with the Development of Erectile Dysfunction Following Radiotherapy for Prostate Cancer.* Oral presentation at the 54th annual ASTRO meeting (2012).

Ko E, Kerns S, Stock R, Stone N, Ostrer H, Rosenstein B. *Association of Genetic Factors with PSA Response in Prostate Cancer Patients Receiving Definitive Radiotherapy.* Poster presented at the 54th annual ASTRO meeting (2012).

Kerns S, Stock R, Stone N, Buckstein M, Shao Y, Campbell C, Rath L, De Ruysscher D, Lammering G, Hixson R, Cesaretti J, Terk M, Ostrer H, Rosenstein B. *A Two-Stage Genome-Wide Association Study to Identify Single Nucleotide Polymorphisms Associated with Development of Erectile Dysfunction Following Radiotherapy for Prostate Cancer.* International journal of radiation oncology, biology, physics. 2012 Sep 25. doi: 10.1016/j.ijrobp.2012.08.003. [Epub ahead of print].

Kerns S, Stone N, Stock R, Rath L, Ostrer H, Rosenstein B. *A Two-Stage Genome-Wide Association Study to Identify Single Nucleotide Polymorphisms Associated with Change in American Urological Association Symptom Score Following Radiotherapy for Prostate Cancer*. Manuscript in preparation.

Genome Wide Association Study to Identify Genetic Variants Associated with Urinary Symptoms Following Radiotherapy for Prostate Cancer

Kerns S, Stock R, Stone N, Ostrer H, Rosenstein B

Purpose/Objectives: Brachytherapy and external beam radiation achieve high cure rates for prostate adenocarcinoma. Though treatment delivery has improved over time, many patients still experience some form of late urinary symptoms that significantly impact quality of life. Even after controlling for clinical factors, considerable variability in toxicity is observed suggesting a genetic component. A predictive tool including genetic factors would assist in personalizing treatment. We performed a two-stage genome wide association study (GWAS) to identify genetic factors associated with urinary morbidity following radiotherapy for prostate cancer.

Methods: Prostate cancer patients treated with brachytherapy alone or brachytherapy plus external beam radiation therapy (EBRT) were assessed for urinary morbidity as measured by change in International Prostate Symptom Score (IPSS) from baseline. A total of 783 patients who had baseline IPSS available and > 1 year of follow-up were included. The change in IPSS was assessed at each 6-month follow-up interval between 1 year and 5 years post-treatment and evaluated as a quantitative trait in genetic association tests. Genotyping was done in two stages with patients split randomly into a discovery cohort (N=347) and a replication cohort (N=436). The discovery cohort was genotyped for ~900,000 SNPs using Affymetrix v6.0 arrays. The 1,480 SNPs most strongly associated with urinary morbidity were then selected for genotyping in the replication cohort using an Illumina custom array. Multivariate linear regression was used to test for association between each SNP and change in IPSS while controlling for pre-treatment IPSS, hypertension and race/ethnicity. Four different genetic inheritance models were investigated for each SNP: allelic, genotypic, dominant and recessive. Combined p-values were calculated for the discovery and replication studies using Fisher's method after filtering on agreement in effect direction.

Results: Several genomic regions were identified that contained clusters of SNPs with combined p-values reaching significance (1E-05 after correction for multiple comparisons). Interestingly, some of the significant SNPs were more strongly associated with early onset of urinary morbidity (between 1 year and 3 years post-treatment), whereas other significant SNPs showed a stronger association with later onset of urinary morbidity (between 3 years and 5 years post-treatment).

Conclusions: This study identifies several potential predictive genetic variants that are associated with urinary morbidity following prostate radiotherapy and could potentially be used to predict the severity of urinary symptoms for individuals receiving radiotherapy for prostate cancer. This work was supported by grants PC074201 from the DOD Prostate Cancer Research Program and 1R01CA134444 from NIH.

Genome Wide Association Study to Identify Genetic Variants Associated with the Development of Erectile Dysfunction Following Radiotherapy for Prostate Cancer

Buckstein M, Kerns S, Stock R, Stone N, Ostrer H, Rosenstein B

Purpose/Objectives: Brachytherapy and external beam radiation therapy (EBRT) afford high rates of local control for prostate adenocarcinoma but carry the risk of late toxicities including erectile dysfunction (ED). When controlling for treatment characteristics, considerable variability in toxicity is observed suggesting a genetic component. A predictive tool including genetic factors would assist in weighing the benefits of radiation with the risks of chronic side effects. We performed a two-stage genome wide association study (GWAS) to identify genetic factors predictive for developing ED.

Methods: Prostate cancer patients treated with brachytherapy alone or brachytherapy plus EBRT were genotyped and selected for development of ED. ED was evaluated using the Sexual Health Inventory for Men (SHIM) questionnaire administered before treatment and during follow-up every 6 months. Patients were required to be potent prior to treatment (SHIM ≥ 16) and have ≥ 1 year follow up. Androgen Deprivation Therapy was allowed, but patients with persistent castrate-level testosterone were excluded. ED cases were defined by any post-treatment SHIM ≤ 7, and controls were defined by post-treatment SHIM≥ 16 for all follow-up visits up to 5 years post-treatment. Genotyping was done in two stages with patients split randomly into a discovery cohort (132 cases and 103 controls) and a replication cohort (128 cases and 102 controls). From the results of the discovery GWAS in which ~900,000 SNPs were genotyped using an Affymetrix v6.0 array, 930 SNPs most strongly associated with ED were selected for follow-up genotyping in the replication cohort using an Illumina prostate custom array. Multivariate logistic regression was used to test for association between each SNP and ED while controlling for age, hormone use, EBRT, and race/ethnicity. Four different genetic inheritance models were investigated for each SNP: allelic, genotypic, dominant and recessive. Combined p-values were calculated for the discovery and replication studies using Fisher's method after filtering on agreement in effect direction.

Results: We identified 5 genes possessing a total of 8 SNPs that each exhibited a combined p-value, using the discovery and replication cohorts, less than 10^{-4} for association with ED. The combined odds ratios for these SNPs range from 1.99 (95% CI 1.45 – 2.74) for the SNP with the smallest effect to 3.16 (95% CI 1.89 – 5.29) for the SNP with the largest effect.

Conclusions: This study identifies several potential predictive genetic variants that are associated with ED following prostate radiotherapy. This work was supported by grants PC074201 from the DOD Prostate Cancer Research Program and 1R01CA134444 from NIH.

Association of Genetic Factors with PSA Response in Prostate Cancer Patients Receiving Definitive Radiotherapy

Ko E, Kerns S, Stock R, Stone N, Ostrer H, Rosenstein B

Purpose/Objectives: Following definitive radiotherapy for prostate cancer, patients that attain a rapid response to a PSA nadir have been shown to have significantly better long-term clinical outcomes. Aside from treatment parameters, genetic factors are hypothesized to influence post-treatment PSA outcomes. We utilized a two-stage genome-wide association study (GWAS) to identify single nucleotide polymorphisms (SNPs) associated with time to PSA nadir.

Methods: We identified a cohort of 345 patients with low and intermediate risk prostate cancer who received brachytherapy with or without external beam radiation therapy between 1994-2008. None of these patients received androgen-deprivation therapy. In our two-stage analysis, patients who achieved PSA nadir (defined as PSA<0.1, <0.2, <0.3, or <0.5ng/ml) were randomly assigned to the discovery (n=170) or validation (n=175) cohorts, with equal weighting of pretreatment and treatment variables known to be associated with PSA outcomes. In the discovery phase, 900,000 SNPs were genotyped using an Affymetrix v6.0 array, and multivariate linear regression was used to test for associations between these SNPs and time to PSA nadir, while controlling for race/ethnicity. In the validation phase, a parallel multivariate linear regression was performed with a subset of 398 SNPs genotyped with an Illumina prostate custom array. Four different genetic inheritance models were tested for each SNP: allelic, genotypic, dominant and recessive. Combined p-values were calculated using Fisher's method.

Results: Median follow up for all patients was 75mos (range 10-215mos). 95%, 90%, 85%, and 72% of patients in the discovery cohort and 94%, 90%, 82%, and 73% of patients in the validation cohort attained a PSA nadir of <0.5, <0.3, <0.2, and <0.1ng/ml, respectively. Median post-treatment intervals to attain these PSA nadirs were comparable between cohorts and were 20mos(range 0.6-116mos), 28mos (range 0.6-116mos), 34mos (range 0.6-116mos), and 41mos (range 3-118mos), respectively. In combined analysis of the discovery and validation cohorts, we identified several SNPs that were significantly associated with a rapid interval to PSA nadir (combined p-values 10^-7 to 10^-4). In multivariate analysis with pretreatment (initial PSA, clinical stage, Gleason score) and treatment (BED) covariates, the identified SNPs were independently predictive of interval to PSA nadir.

Conclusions: We identified a panel of candidate SNPs that were strongly associated with time to PSA nadir following definitive prostate radiotherapy. Since the time to PSA nadir has been shown to be significantly associated with long-term clinical outcomes (e.g., freedom from biochemical failure and distant metastasis), our results suggest that at least some of these SNPs may be prognostically useful in the setting of prostate radiotherapy. This work was supported by grants PC074201 from the DOD Prostate Cancer Research Program and 1R01CA134444 from NIH.

A Two-Stage Genome-Wide Association Study to Identify Single Nucleotide Polymorphisms Associated with Development of Erectile Dysfunction Following Radiotherapy for Prostate Cancer*

Sarah L. Kerns, Ph.D.,M.P.H.[1,4], Richard Stock, M.D.[1], Nelson Stone, M.D.[1,2], Michael Buckstein, M.D.,Ph.D.[1], Yongzhao Shao, Ph.D.[3], Christopher Campbell, B.S.[4], Lynda Rath, B.F.A.[1], Dirk DeRuysscher, M.D.,Ph.D.[5], Guido Lammering, M.D.,Ph.D.[5], Rosetta Hixson, M.D.[6], Jamie Cesaretti, M.D.[6], Mitchell Terk, M.D.[6], Harry Ostrer, M.D.[4,**], Barry S. Rosenstein, Ph.D.[1,7,8,**]

1. Department of Radiation Oncology, Mount Sinai School of Medicine, New York, NY
2. Department of Urology, Mount Sinai School of Medicine, New York, NY
3. Division of Biostatistics, New York University School of Medicine, New York, NY
4. Departments of Pathology and Genetics, Albert Einstein College of Medicine, Bronx, NY
5. Department of Radiation Oncology, Maastricht University Medical Center, Maastricht, the Netherlands
6. Florida Radiation Oncology Group, Jacksonville, FL, United States
7. Department of Radiation Oncology, New York University School of Medicine, New York, NY
8. Departments of Dermatology and Preventive Medicine, Mount Sinai School of Medicine, New York, NY

*Collaboration developed under the framework of the Radiogenomics Consortium
**Equal contributions as senior authors to this manuscript
Corresponding author: Barry Rosenstein, Ph.D., Department of Radiation Oncology, Atran Laboratory Building, Room 206, 1428 Madison Avenue, New York, NY 10029.
Tel: 212-241-9408, Fax: 212-996-8927, barry.rosenstein@mssm.edu.

This research was supported by grants RSGT-05-200-01-CCE from the American Cancer Society, PC074201 from the Department of Defense and 1R01CA134444 from the National Institutes of Health.

Purpose

To identify single nucleotide polymorphisms (SNPs) associated with development of erectile dysfunction (ED) among prostate cancer patients treated with radiotherapy.

Methods and Materials

A two-stage genome-wide association study (GWAS) was performed. Patients were split randomly into a stage I discovery cohort (132 cases, 103 controls) and a stage II replication cohort (128 cases, 102 controls). The discovery cohort was genotyped using Affymetrix 6.0 genome-wide arrays. The 940 top ranking SNPs selected from the discovery cohort were genotyped in the replication cohort using Illumina iSelect custom SNP arrays.

Results

12 SNPs identified in the discovery cohort and validated in the replication cohort were associated with development of ED following radiotherapy (Fisher combined p-values $2.1x10^{-5}$ to $6.2x10^{-4}$). Notably, these 12 SNPs lie in or near genes involved in erectile function or other normal cellular functions (adhesion and signaling) rather than DNA damage repair. In a multivariable model including non-genetic risk factors, the odds ratios for these SNPs ranged from 1.6 to 5.6 in the pooled cohort. There was a striking relationship between the cumulative number of SNP risk alleles an individual possessed and ED status (Sommers' D p-value = $1.7x10^{-29}$). A one-allele increase in cumulative SNP score increased the odds for developing ED by a factor of 2.2 (p-value = $2.1x10^{-19}$). The cumulative SNP score model had a sensitivity of 84% and specificity of 75% for prediction of developing ED at the radiotherapy planning stage.

Conclusions

This GWAS identified a set of SNPs that are associated with development of ED following radiotherapy. These candidate genetic predictors warrant more definitive validation in an independent cohort.

Key Words: prostate cancer, genetic predictors, late effects, brachytherapy

Summary

Through a two-stage genome wide association study, 12 SNPs were identified that were associated with the development of ED following radiation treatment for prostate cancer. If validated further, these SNPs could provide the basis for an assay that would enable radiation oncologists to more accurately predict which men are most likely to develop ED following prostate cancer radiotherapy.

Introduction:

Prostatectomy, brachytherapy, and external beam radiation therapy (EBRT) as treatments of prostate adenocarcinoma offer excellent rates of long-term disease-free survival[1]. Thus, patients and clinicians consider risk of short and long term side effects for choice of treatment. These toxicities include genitourinary, gastrointestinal, and erectile dysfunction (ED).

Radiation therapy results in favorable erectile function preservation. Brachytherapy with or without EBRT has a 57.9% overall potency preservation rate at 10 years and up to 80% in selected men[2]. Similar results have been reported for brachytherapy[3-4]. Treatment-related factors that increase the risk for ED include age, pretreatment potency, use of androgen deprivation therapy (ADT), radiation type, dose, and comorbidities[2,5-6]. Even when controlling for these factors, significant variation exists for developing ED suggesting an intrinsic radiation sensitivity genetic risk. This hypothesis is supported by SNP association studies performed by ourselves and others[7-8].

To broaden the search for genetic factors predictive for ED development in men treated with brachytherapy and/or EBRT, we performed a two-stage GWAS. This analysis identified 12 new candidate SNPs.

Methods and Materials:

Patient Characteristics:

Men treated with definitive radiation for biopsy proven adenocarcinoma of the prostate were recruited from Mount Sinai Medical Center (MSMC) and Florida Radiation Oncology Group (FROG). This study was approved by the Institutional Review Board of MSMC. All patients provided informed consent. Radiation consisted of brachytherapy alone with a full ^{125}Iodine implant prescribed to 160Gy (TG43), a partial ^{103}Palladium implant prescribed to 100Gy followed in 6–8 weeks by external beam irradiation (3D conformal or image guided intensity modulated radiation therapy, IMRT) to 24 to 50Gy (median 45Gy), or EBRT alone to 66.6–81Gy (IMRT). Approximately 95% of implants were performed by or under the direct supervision of a single physician (RS). The remaining 5% were performed by providers at MSMC using the same treatment algorithm (1.8 Gy fractions to 45 Gy using image guided IMRT). Delivered radiation doses were converted to the biologically effective dose (BED) as described previously[9].

Patients were followed prospectively every 3-6 months with the patient-administered Sexual Health Inventory for Men (SHIM) questionnaire[10]. Patients treated prior to introduction of the SHIM questionnaire (42%), were assessed using the Mount Sinai Erectile Function (MSEF) score (0=no erections; 1=erections but insufficient for intercourse; 2=erections suboptimal but sufficient for intercourse; 3=optimal erections), which correlates with SHIM [11]. Included patients (N=593) were potent before treatment (SHIM$\geq$ 16 or MSEF $\geq$ 2), and have $\geq$1 year follow up. ADT was administered for 3-6 months in low risk patients with prostate volumes >50cc, in intermediate risk patients for 6 months with brachytherapy monotherapy or for 9-24 months in high risk patients with combination brachytherapy and EBRT. Patients with testosterone levels <50ng/dl at one-year post treatment were excluded (n=5). ED was assessed using SHIM scores reported between 1 and 5 years post-treatment. Cases were defined by at least one post-treatment SHIM $\leq$ 7 ("severe ED"), and contr ols were defined by all post-treatment SHIM $\geq$ 16 ("no" or "mild" ED) with or without use of a phosphodiesterase type 5 inhibitor.

260 patients met the ED case definition and 205 met the ED control definition, and were included in the GWAS. Cases were significantly older than controls at time of treatment (65.7 years vs. 60.3 years, p<0.001), were significantly more likely to have received ADT (60.8% vs. 31.5%, p<0.001), had significantly larger prostate volume at time of treatment (48.1mm^3 vs. 44.4 mm^3; p=0.048), and were significantly more likely to have been treated with combination implant + EBRT (53.5% vs. 35.1%, p<0.001) (Table 1). Gleason score was higher in cases than controls, but because Gleason score correlates with treatment modality, it was omitted from analyses.

A small group of prostate cancer patients (n=55) was recruited from the Maastricht Radiation Oncology clinic and utilized as a test cohort. Patients were treated with EBRT alone prescribed to 68-72Gy in 2Gy fractions or with brachytherapy alone prescribed to 145Gy. A unique questionnaire for measuring ED was utilized in this group. The 4 elements of this questionnaire which correlated directly to the SHIM were utilized to define cases and controls based on post-treatment score after excluding patients who had a low pre-treatment score.

Genotyping:

Genomic DNA was isolated from lymphocytes. Discovery cohort DNA samples were genotyped for ~909,000 SNPs using Affymetrix v6.0 arrays (Affymetrix, Santa Clara, CA), and genotypes were called using Genotyping Console. Replication cohort DNA was genotyped using Illumina iSelect custom SNP arrays (Illumina, San Diego, CA), and genotypes were called by GenomeStudio. SNPs were excluded from analysis if missing in >5% of samples (137,589 SNPs in the discovery dataset; 22 SNPs in the replication dataset) or if they had minor allele frequency <5% (157,580 SNPs in the discovery dataset). Individuals were excluded if they showed cryptic relatedness as assessed by pairwise identity-by-state (8 pairs) or if they had call rate <90% (N=2). Duplicate control samples included in both rounds of genotyping showed >95% concordance. Three control samples (a trio of two parents and an offspring) were included on both the Affymetrix and Illumina genotyping platforms and showed >99% concordance across platforms. PLINK was used to perform QC and association tests[12].

Principal components analysis (PCA) was performed using 11 reference populations from the International HapMap Project[13]. PCA was performed using a random 100,000 SNPs in the discovery study and 860 ancestry-informative SNPs in the replication study. SNP data processing and PCA was carried out using R 2.13.1[14].

Statistical Analysis:

Association between non-genetic variables and ED was assessed using chi-square tests or analysis of variance. SNP association tests were carried out using multivariable logistic regression. Combined p-values for the discovery and replication cohorts were calculated using Fisher's method after filtering for agreement in odds ratio direction and inheritance model (out of additive, genotypic, dominant and recessive). Top SNPs were examined for their utility in predicting ED following radiotherapy using multivariate logistic regression models inclusive of age, ADT, treatment, and PCs. The area under the ROC curve was used as a measure of the discriminatory power. IBM SPSS Statistics 19 was used for univariate and multivariate analysis.

Results:

A two-stage GWAS was performed to identify SNPs associated with ED (Figure 1). After QC, the discovery cohort included 132 cases and 103 controls genotyped for 614,453 SNPs with a call rate of ~99%. In agreement with self-reported race/ethnicity (Table 1), PCA confirmed that the majority of the patients were of European ancestry, with approximately 25% of individuals showing African, Hispanic and/or Asian ancestry. Inclusion of the first five PCs in association tests adequately controlled for ancestry as evidenced by a low genomic inflation factor (1.02 after correction compared to 1.11 before correction). Multivariable logistic regression was performed to test the association for each of the 614,453 SNPs with ED status while controlling for PCs and non-genetic factors associated with ED on univariate analysis (age, ADT, and treatment; Table 1). 940 SNPs from ~550 genomic regions had p-values from $8x10^{-4}$ to $2x10^{-6}$ and were genotyped in the replication cohort.

The replication cohort included 128 cases and 102 controls genotyped successfully (call rate >99%) for 918 SNPs. After adjusting for age, ADT, treatment, and PCs, 21 SNPs were associated with ED in the replication cohort (p-value < 0.10 and agreement in inheritance model and odds ratio direction). These 21 SNPs had Fisher-combined p-values between $9x10^{-4}$ and $2x10^{-5}$ (Table 2). Four additional SNPs had combined p-value between $1x10^{-4}$ and $2x10^{-5}$ but replication p-values >0.1. These SNPs are clustered within one genomic region suggesting a true association, and were included in downstream analysis. We ran a re-sampling procedure to reduce the list to the most robustly associated SNPs. We randomly split the pooled cohorts into two equal groups and calculated separate p-values and Fisher combined p-values. After 1,000 iterations, we identified 12 of the 25 SNPs with median Fisher p-value $<1x10^{-3}$ (Table 2). In the case where multiple SNPs were in linkage disequilibrium, we selected one from the cluster.

Table 3A shows the odds ratios associated with each of these 12 SNPs in the full cohort (N = 465) in a multivariable model with age, ADT, treatment and PCs. The odds ratios for the individual SNPs ranges from 1.6 to 5.6 indicating that after controlling for clinical factors, the SNPs represent clinically relevant predictors of ED. Though this was a multi-ethnic cohort, it is important to note that PCs were not significantly associated with ED, suggesting that ancestry was not a major confounder. The model including SNPs and non-genetic variables was a better predictor of ED compared to the model with age, ADT and treatment alone (AUCs of 0.89 and 0.75 respectively) (Figure 2). The AUC in the full cohort is likely an over-estimate because the model was being tested in the same patients from which it was developed. The AUC from the replication cohort alone (0.85) is likely closer to the true value, but the model should be tested further in additional large, independent cohorts.

When these 12 SNPs were combined into a cumulative SNP score representing a quantitative measure of risk alleles possessed by each individual, a one-allele increase in cumulative SNP score increased the odds of developing ED by a factor of 2.2 (95% CI 1.9,2.6; p-value = $2.1x10^{-19}$) after controlling for clinical factors and ancestry (Table 3B). We investigated the effect of including BED and prostate volume in the multi-variable model rather than treatment modality. These factors did not have a substantial effect on the odds ratios for the cumulative SNP score (Table 3C). Using ordered logistic regression, the cumulative SNP score was also a

significant predictor of SHIM category[15] (OR=1.5, 95%CI 1.4-1.7; Supplemental Table 1). The ordered logistic regression model correctly assigned 52.4% of the patients to their SHIM category; just 4.3% of individuals were grossly misclassified (i.e. group 1 classified as group 5 and vice versa). Furthermore, there was a striking dose-dependent relationship between number of risk alleles and ED case status (Sommers' D p-value for directional association = $1.7x10^{-29}$) (Table 4A). The multivariable model including cumulative SNP score, age, ADT, treatment, and PCs has sensitivity of 83.7% and specificity of 74.6%.

Alemozaffar et al. recently developed validated models to predict erectile function at 2 years following radiation therapy[16]. Among patients who selected brachytherapy for treatment, age, pre-treatment function, race/ethnicity and body-mass index were significant predictors of erectile function (AUC=0.90). Among patients who selected EBRT, PSA level, pre-treatment function and ADT were significant predictors of erectile function (AUC=0.87). We sought to determine how well these models fit our cohort, and whether adding the SNP score improves the AUC. Since our cohort includes a large proportion of patients treated with combination brachytherapy with EBRT, we combined the elements of the two models from Alemozaffar et al, excluding BMI, which was not available. This model achieved an AUC of 0.80 in our cohort. Addition of the SNP score (dichotomozied at the median of 9 risk alleles) improved the AUC to 0.85. These AUCs would likely increase with addition of BMI.

To show a simplified understanding of what the SNPs could mean in the context of age alone, we stratified the pooled cohort by age (using the median, 62, as a cut-off) and cumulative SNP score (using the median, 9, as a cut-off). Approximately 21.8% of younger men with $\leq$ 9 risk alleles developed ED compared to 52.7% of younger men with > 9 risk alleles (Table 4B). Conversely, 54.8% of older men with $\leq$ 9 risk alleles developed ED compared to 92.5% of older men with > 9 risk alleles. Stated more generally, in this cohort, a younger man with more risk alleles has roughly the same likelihood of developing ED as an older man with fewer risk alleles.

We tested the model in two independent, small cohorts of patients treated with radiotherapy and followed for development of ED. The cohort from the Maastricht Radiation Oncology clinic was comprised of 55 individuals (38 cases and 17 controls) treated with either EBRT alone (59%) or brachytherapy alone (41%). The AUC for the model including SNPs and non-genetic variables in this cohort was 0.79. The second test cohort comprised 66 individuals of African American ancestry from our previously published GWAS[8]. In this cohort, the model produced an AUC of 0.81. Though these cohorts were under-powered to expect significant SNP-phenotype associations, they provide an indicator of overall model performance.

Finally, we investigated the top SNPs from the previously published GWAS of post-radiotherapy ED in an African American cohort. Seven of the top 31 SNPs from that study have p-values < 0.05 in the cohort from the current study, though none of the SNPs reached genome-wide significance (Supplementary Table 2). This is not surprising since the SNPs identified in the African American study are rare in European Americans[8]. To better attempt to replicate the regions tagged by these SNPs in the current study, we scanned 150kb upstream and downstream of each SNP identified in the African American GWAS, and identified 5 regions that contain at least one SNP with a low p-value ($<1x10^{-2}$) (Supplementary Table 3). Because we searched a limited number of regions with *a priori* evidence for association with ED, a genome-wide level of significance may be too strict, and some of these regions may represent true positives.

Discussion:

We identified 25 SNPs that showed low p-values, with effects of similar magnitude and in the same direction in both discovery and replication cohorts. Using re-sampling, we reduced the complexity of this list to 12 SNPs. Although none of these SNPs met a strict genome-wide significance p-value threshold of 10^{-7} to 10^{-8}, they may represent true associations as evidenced by their increased accuracy for risk prediction compared with non-genetic variables alone for the case, control and intermediate groups. The AUC achieved by the model

developed from this study is likely to be an overestimate as it was tested on the pooled discovery and replication cohorts from which it was developed. Still, this increased accuracy was observed in two small independent groups of cases and controls from the MSMC cohort and the African American cohort previously studied. The utility of these SNPs for predicting risks was shown not only in the multi-SNP model, but also in a model of additive allelic risk.

There are several limitations to note. First, none of the individual SNPs reached genome-wide significance. Other studies that did not achieve genome-wide significance have observed replication of suggestive SNPs however[17]. Thus, it will be important to investigate the SNPs identified in this study further in a large, independent cohort of similarly treated patients. A second limitation is that data on co-morbidities was limited. While we found no significant association between ED and smoking or diabetes as assessed at time of diagnosis, these factors could have changed over the course of follow-up. It is possible that some proportion of the risk for ED is due to worsening co-morbid conditions, which could modify the effects of the SNP-phenotype association. A third limitation is that more extensive analysis of dose-volume parameters could not be adequately assessed in this cohort because of the relatively uniform treatment received by the patients.

Although this study and our first GWAS on ED following radiation therapy for prostate cancer were similar, the ethnic make-up of the two cohorts was quite different[8]. The first study was comprised of African-American men, whereas this study was composed predominantly of men of European ancestry. When comparing the results of these two studies, most of the top 31 SNPs from the first study did not have low p-values. This result was expected, because the SNPs we identified as associated with ED in men of African ancestry are almost universally rare in European populations. Yet, part of the same repertoire of genes may be involved, because 11 out of the top 31 genes in the current study had at least one SNP within 150kb of the SNP associated with ED in African-Americans with p-values $< 10^{-2}$.

As with the previous study, some of the SNPs identified in this study lie in or near genes that may encode biological activities involved in erectile function rather than DNA damage repair – the focus of earlier studies. For example, the 17-beta-hydroxysteroid dehydrogenase II gene (HSD17B2) catalyzes the oxidative metabolism of androgens and estrogens in human peripheral tissues[18]. Other physiological functions included cell adhesion and cell matrix association (CDCP1)[19], glutaredoxin oxidoreductase enzyme activity (GLRX3)[20], and mineralocorticoid receptor signaling in response to aldosterone (CNKSR3)[21]. The Ingenuity Pathway Analysis software (Ingenuity Systems, Inc., Redwood City, CA) grouped 11 of these 12 genes into a common pathway containing direct or indirect connections between each other. This pathway contains genes involved in cellular growth and proliferation, cell-cell signaling and tissue development. The SNPs associated with ED may encode sensitivity to radiation injury through these various pathways.

It is important to note that the markers identified in this study are tag SNPs indirectly associated with ED. Higher density genotyping and/or sequencing could identify more predictive SNPs or rare variants that are in linkage disequilibrium and may produce direct effects on protein expression, modification, or transcriptional regulation.

References:

1. Grimm P, Billiet I, Bostwick D, et al. Comparative analysis of prostate-specific antigen free survival outcomes for patients with low, intermediate and high risk prostate cancer treatment by radical therapy. Results from the Prostate Cancer Results Study Group. *BJU Int.* Feb 2012;109 Suppl 1:22-29.

2. Snyder KM, Stock, Richard G., Buckstein, Michael, Stone, Nelson N. Long-term potency preservation following brachytherapy for prostate cancer. *BJU Int.* 2012;10.1111/j.1464-410X.2011.10800.x.

3. Merrick GS, Butler WM, Wallner KE, et al. Erectile function after prostate brachytherapy. *International journal of radiation oncology, biology, physics.* Jun 1 2005;62(2):437-447.
4. Potters L, Torre T, Fearn PA, Leibel SA, Kattan MW. Potency after permanent prostate brachytherapy for localized prostate cancer. *International journal of radiation oncology, biology, physics.* Aug 1 2001;50(5):1235-1242.
5. Robinson JW, Moritz S, Fung T. Meta-analysis of rates of erectile function after treatment of localized prostate carcinoma. *International journal of radiation oncology, biology, physics.* Nov 15 2002;54(4):1063-1068.
6. Talcott JA, Manola J, Clark JA, et al. Time course and predictors of symptoms after primary prostate cancer therapy. *J Clin Oncol.* Nov 1 2003;21(21):3979-3986.
7. Rosenstein BS. Identification of SNPs associated with susceptibility for development of adverse reactions to radiotherapy. *Pharmacogenomics.* Feb 2011;12(2):267-275.
8. Kerns SL, Ostrer H, Stock R, et al. Genome-wide association study to identify single nucleotide polymorphisms (SNPs) associated with the development of erectile dysfunction in African-American men after radiotherapy for prostate cancer. *International journal of radiation oncology, biology, physics.* Dec 1 2010;78(5):1292-1300.
9. Stock RG, Stone NN, Cesaretti JA, Rosenstein BS. Biologically effective dose values for prostate brachytherapy: effects on PSA failure and posttreatment biopsy results. *International journal of radiation oncology, biology, physics.* Feb 1 2006;64(2):527-533.
10. Rosen RC, Cappelleri JC, Smith MD, Lipsky J, Pena BM. Development and evaluation of an abridged, 5-item version of the International Index of Erectile Function (IIEF-5) as a diagnostic tool for erectile dysfunction. *Int J Impot Res.* Dec 1999;11(6):319-326.
11. Zagar TM, Stock RG, Cesaretti JA, Stone NN. Assessment of postbrachytherapy sexual function: a comparison of the IIEF-5 and the MSEFS. *Brachytherapy.* Jan-Mar 2007;6(1):26-33.
12. Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet.* Sep 2007;81(3):559-575.
13. Consortium IH. The International HapMap Project. *Nature.* Dec 18 2003;426(6968):789-796.
14. *R: A language and environment for statistical computing* [computer program]. Version 2.13.1. Vienna, Austria.2011.
15. Wheat JC, Hedgepeth RC, He C, Zhang L, Wood DP, Jr. Clinical interpretation of the Expanded Prostate Cancer Index Composite-Short Form sexual summary score. *J Urol.* Dec 2009;182(6):2844-2849.
16. Alemozaffar M, Regan MM, Cooperberg MR, et al. Prediction of erectile function following treatment for prostate cancer. *JAMA.* Sep 21 2011;306(11):1205-1214.
17. Panagiotou OA, Ioannidis JP. What should the genome-wide significance threshold be? Empirical replication of borderline genetic associations. *Int J Epidemiol.* Feb 2012;41(1):273-286.
18. Wu L, Einstein M, Geissler WM, Chan HK, Elliston KO, Andersson S. Expression cloning and characterization of human 17 beta-hydroxysteroid dehydrogenase type 2, a microsomal enzyme possessing 20 alpha-hydroxysteroid dehydrogenase activity. *J Biol Chem.* Jun 15 1993;268(17):12964-12969.
19. Brown TA, Yang TM, Zaitsevskaia T, et al. Adhesion or plasmin regulates tyrosine phosphorylation of a novel membrane glycoprotein p80/gp140/CUB domain-containing protein 1 in epithelia. *J Biol Chem.* Apr 9 2004;279(15):14772-14783.
20. Witte S, Villalba M, Bi K, Liu Y, Isakov N, Altman A. Inhibition of the c-Jun N-terminal kinase/AP-1 and NF-kappaB pathways by PICOT, a novel protein kinase C-interacting protein with a thioredoxin homology domain. *J Biol Chem.* Jan 21 2000;275(3):1902-1909.
21. Ziera T, Irlbacher H, Fromm A, et al. Cnksr3 is a direct mineralocorticoid receptor target gene and plays a key role in the regulation of the epithelial sodium channel. *FASEB J.* Nov 2009;23(11):3936-3946.

Figure 1. Study design.

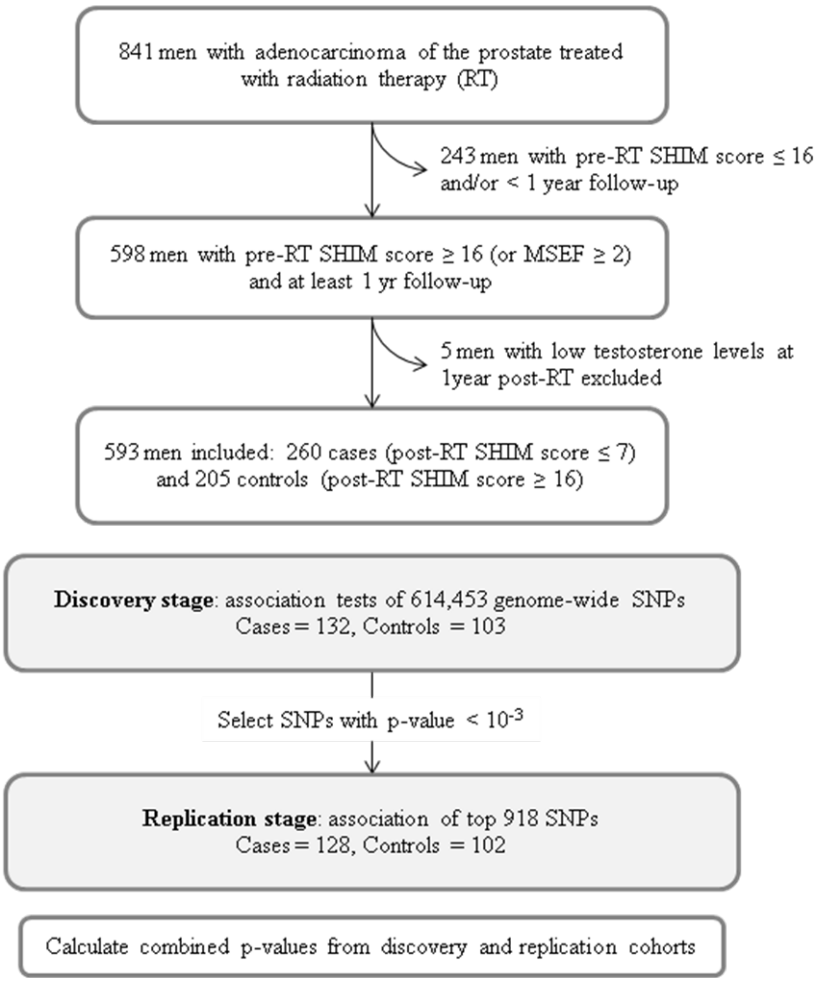


Table 1. Patient characteristics. Bold values are significantly different between cases and controls (p-value <0.05). Ethnicity, smoking status, diabetes, and hypertension are patient-reported.

	Cases ***N = 260***	***Controls*** ***N = 205***	***Intermediate*** ***N = 128***	***Total*** ***N = 593***
Age (yrs), mean(sd)	**66.5 (6.7)**	**60.5 (6.9)**	62.6 (6.1)	63.7 (7.1)
Follow-up (months), mean(sd)	45.1 (14.8)	45.1 (14.4)	47.3 (13.0)	45.6 (14.3)
Hormones, N(%)	**158 (60.8%)**	**72 (35.1%)**	61 (47.7%)	306 (49.0%)
Treatment, N(%)				
Implant Only	**114 (43.8%)**	**128 (62.4%)**	79 (61.7%)	346 (55.4%)
Implant + EBRT	**139 (53.5%)**	**72 (35.1%)**	48 (37.5%)	266 (42.6%)
EBRT Only	**7 (2.7%)**	**5 (2.4%)**	1 (0.8%)	13 (2.1%)
Total BED (Gy2), mean(sd)	205.5 (21.1)	202.8 (22.8)	203.1 (21.4)	202.5 (24.5)
Gleason, N(%)				
≤ 6	**141 (54.2%)**	**146 (71.2%)**	90 (70.3%)	398 (63.7%)
7	**76 (29.2%)**	**47 (22.9%)**	31 (24.2%)	165 (26.4%)
≥ 8	**43 (16.5%)**	**12 (5.9%)**	7 (5.5%)	62 (9.9%)
Prostate Volume at time of diagnosis (mm^3), mean(sd)	**48.1 (21.3)**	**44.4 (14.5)**	47.1 (17.5)	46.6 (18.3)
Self-reported ethnicity, N(%)				
European American	197 (76.4%)	161 (80.5%)	99 (77.3%)	482 (78.9%)
African American	27 (10.5%)	25 (12.5%)	16 (12.5%)	74 (12.1%)
Hispanic	25 (9.7%)	10 (5.0%)	10 (7.8%)	46 (7.5%)
Asian	6 (2.3%)	2 (1.0%)	1 (0.8%)	9 (1.5%)
Smoker, N(%)	113 (43.5%)	78 (38.0%)	55 (43.0%)	255 (40.8%)
Diabetes, N(%)	15 (5.8%)	6 (2.9%)	12 (9.4%)	34 (5.4%)
Hypertension, N(%)	89 (34.2%)	58 (28.3%)	39 (30.5%)	190 (30.4%)

Table 2. SNPs associated with ED following radiation therapy for treatment of prostate cancer. Results are adjusted for age, ADT, treatment, and PCs. Genotypes are reported with the risk allele listed first. Alleles are shown as risk/non-risk. *SNP included in multivariable model; ^ OR is for the homozygous risk genotype relative to the homozygous non-risk genotype.

				Discovery Cohort				***Replication Cohort***					
dbSNPrsID	***Location***	***Alleles***	***Nearest Gene(s)***	***Genotype Cases***	***Genotype Controls***	***OR (95%CI)***	***p-value***	***Genotype Cases***	***Genotype Controls***	***OR (95%CI)***	***p-value***	***Combined p-value***	***Inheritance Model***
rs322895*	1q32.1	C/T	NR5A2/PTPRC	45/70/14	28/38/33	4.7 (2.1,10.6)	$2.2x10^{-4}$	49/64/15	35/47/20	2.2 (1.0,5.2)	$6.0x10^{-2}$	$1.6x10^{-4}$	Recessive
rs1779969	1q44	G/A	AHCTF1	25/82/25	19/47/37	3.4 (1.6,6.9)	$8.8x10^{-4}$	34/70/24	27/45/30	1.9 (0.9,3.8)	$8.6x10^{-2}$	$7.9x10^{-4}$	Recessive
rs13389878	2p25.3	G/A	MYT1L	116/14/2	72/30/1	4.3 (1.9,9.4)	$3.7x10^{-4}$	104/22/2	72/29/1	2.2 (1.1,4.5)	$2.8x10^{-2}$	$1.3x10^{-4}$	Dominant
rs11693002*	2p25.3	C/G	MYT1L	114/15/2	70/32/1	4.2 (1.9,9.2)	$2.8x10^{-4}$	101/25/2	67/34/1	2.5 (1.3,5.1)	$7.9x10^{-3}$	$3.1x10^{-5}$	Dominant
rs1563740	2p13.2	A/T	DYSF/CYP26B1	2/37/91	1/15/86	3.8 (1.7,8.5)	$9.7x10^{-4}$	1/28/99	2/18/82	2.0 (0.9,4.4)	$9.6x10^{-2}$	$9.6x10^{-4}$	Dominant
rs3749191*	3p21.31	G/A	CDCP1	75/49/7	14/59/30	3.8 (2.0,7.2)	$4.3x10^{-5}$	70/46/12	46/46/10	2.0 (1.1,3.7)	$3.3x10^{-2}$	$2.1x10^{-5}$	Dominant
rs11747037*	5q22.3	A/G	KCNN2/YTHDC2	14/42/76	37/48/18	2.1 (1.4,3.3)	$6.9x10^{-4}$	57/58/13	41/45/16	1.5 (0.9,2.4)	$8.3x10^{-2}$	$6.2x10^{-4}$	Additive
rs1896974	6p12.2	C/T	PKHD1	45/59/27	12/70/20	4.1 (1.4,12.6)	$3.1x10^{-4}$	39/67/22	23/51/28	2.9 (1.1,5.4)	$4.5x10^{-2}$	$1.7x10^{-4}$	Genotypic^
rs9382062	6p12.2	G/A	PKHD1	46/59/26	15/68/20	2.6 (1.3,5.0)	$1.4x10^{-3}$	40/66/22	23/51/28	3.0 (1.2,7.8)	$4.2x10^{-2}$	$6.4x10^{-4}$	Genotypic^
rs1548335	6q13	A/G	RIMS1/KCNQ5	81/47/4	44/44/15	2.6 (1.6,4.4)	$2.9x10^{-4}$	76/41/11	54/36/12	1.5 (0.9,2.3)	$1.1x10^{-1}$	$3.5x10^{-4}$	Additive
rs6557362*	6q25.2	T/A	CNKSR3	37/59/36	12/44/47	2.2 (1.4,3.5)	$3.7x10^{-4}$	22/64/42	11/53/38	1.5 (0.9,2.4)	$7.9x10^{-2}$	$3.3x10^{-4}$	Additive
rs766838*	7p15.2	G/A	NFE2L3/NPVF	45/70/13	29/39/31	4.4 (1.9,10.2)	$4.2x10^{-4}$	43/65/20	28/47/26	1.9 (0.9,4.1)	$9.4x10^{-2}$	$4.4x10^{-4}$	Recessive
rs1486147*	7p12.3	C/T	TNS3/IGFBP3	27/46/59	4/42/56	8.3 (2.4,28.3)	$7.1x10^{-4}$	16/49/63	8/38/56	2.7 (0.9,7.8)	$7.3x10^{-2}$	$5.7x10^{-4}$	Recessive
rs1397294	8p22	G/C	SGCZ	52/67/12	39/40/24	4.3 (1.8,10.3)	$8.3x10^{-4}$	52/63/13	27/55/20	2.5 (1.1,6.2)	$4.0x10^{-2}$	$3.7x10^{-4}$	Recessive
rs16902486	8q24.21	G/C	PVT1	124/6/2	87/15/0	7.2 (2.1,23.9)	$1.4x10^{-3}$	120/7/1	88/14/0	3.8 (1.2,12.1)	$2.2x10^{-2}$	$3.4x10^{-4}$	Dominant
rs7866508	9q31.3	G/A	PTPN3	120/9/0	77/21/0	5.2 (2.0,13.9)	$9.1x10^{-4}$	118/9/1	86/15/1	2.6 (1.0,7.1)	$6.1x10^{-2}$	$6.0x10^{-4}$	Dominant
rs11017104	10q26.3	A/G	GLRX3	116/13/1	64/36/2	5.9 (2.7,13.1)	$1.1x10^{-5}$	101/25/2	74/27/1	1.5 (0.7,3.0)	$2.5x10^{-1}$	$3.6x10^{-5}$	Dominant
rs10829695	10q26.3	G/A	GLRX3	118/13/1	66/35/2	5.8 (2.6,12.8)	$1.4x10^{-5}$	101/25/1	74/27/1	1.5 (0.8,3.1)	$2.2x10^{-1}$	$4.1x10^{-5}$	Dominant
rs10764930*	10q26.3	G/A	GLRX3	118/13/1	65/36/2	5.9 (2.7,13.1)	$9.3x10^{-6}$	101/25/2	74/27/1	1.5 (0.7,3.0)	$2.5x10^{-1}$	$3.2x10^{-5}$	Dominant
rs10829696	10q26.3	G/A	GLRX3	118/13/1	65/36/1	5.7 (2.6,12.7)	$1.5x10^{-5}$	101/25/2	74/27/1	1.5 (0.7,3.0)	$2.5x10^{-1}$	$5.0x10^{-5}$	Dominant
rs895255*	11q14.1	C/A	SYTL2/CCDC83	68/57/7	47/35/21	5.8 (2.1,15.0)	$5.7x10^{-4}$	64/55/9	45/40/17	2.8 (1.0,7.8)	$4.6x10^{-2}$	$3.0x10^{-4}$	Recessive
rs9595967*	13q14.2	C/G	CYSLTR2	49/65/16	24/49/26	5.0 (2.1,11.8)	$3.0x10^{-4}$	48/61/19	28/47/27	1.9 (0.9,4.2)	$8.5x10^{-2}$	$2.9x10^{-4}$	Recessive
rs11648233*	16q23.3	C/A	HSD17B2	50/58/19	30/43/29	2.2 (1.4,3.6)	$9.2x10^{-4}$	49/58/21	30/42/30	1.8 (1.2,2.8)	$7.7x10^{-3}$	$9.1x10^{-5}$	Additive
rs4794940	17q11.2	A/G	ACCN1	23/67/39	9/45/48	2.4 (1.5,4.0)	$4.3x10^{-4}$	24/60/44	13/49/40	1.5 (0.9,2.3)	$9.2x10^{-2}$	$4.4x10^{-4}$	Additive
rs7245988*	19q13.43	G/A	NLRP11	1/41/89	0/12/90	4.65 (2.1,10.5)	$2.2x10^{-4}$	1/31/96	2/12/88	2.0 (0.9,4.4)	$1.0x10^{-1}$	$2.6x10^{-4}$	Dominant

Table 3. Multivariable logistic regression models with (A) individual SNPs, (B) and (C) cumulative SNP score. Odds ratios for SNPs and SNP score are adjusted for non-genetic factors listed and PCs. *Reference category is "implant only".

A.

Variable	*OR (95%CI)*	*p-value*
rs11693002	2.6 (1.4,5.0)	$2.8x10^{-3}$
rs3749191	2.5 (1.5,4.3)	$7.8x10^{-4}$
rs10764930	2.0 (1.1,3.7)	$3.4x10^{-2}$
rs11648233	2.0 (1.4,3.0)	$2.7x10^{-4}$
rs322895	2.8 (1.4,5.6)	$4.3x10^{-3}$
rs895255	5.6 (2.3,14.0)	$1.9x10^{-4}$
rs1486147	5.5 (2.0,15.2)	$1.1x10^{-3}$
rs7245988	3.4 (1.7,6.8)	$5.7x10^{-4}$
rs9595967	2.3 (1.2,4.6)	$1.2x10^{-2}$
rs6557362	1.6 (1.1,2.4)	$1.1x10^{-2}$
rs766838	2.2 (1.2,4.3)	$1.6x10^{-2}$
rs11747037	1.8 (1.2,2.6)	$4.5x10^{-3}$
Age	1.2 (1.1,1.2)	$8.4x10^{-15}$
ADT	1.9 (1.0,3.4)	$3.7x10^{-2}$
EBRT Only*	0.3 (0.0,1.3)	0.11
Implant + EBRT*	1.9 (1.0,3.4)	$4.1x10^{-2}$

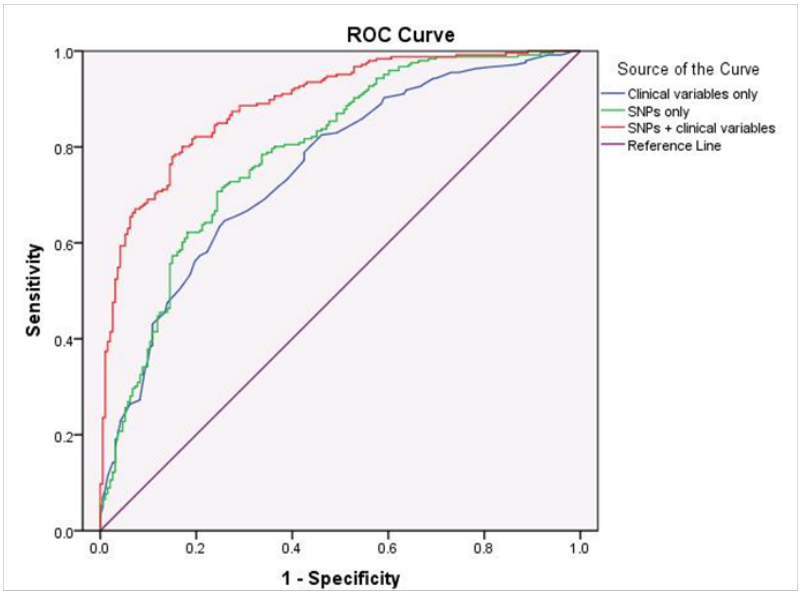
B.

Variable	*OR (95%CI)*	*p-value*
Cumulative SNP score	2.2 (1.8,2.6)	$2.1x10^{-19}$
Age	1.2 (1.1,1.2)	$3.1x10^{-15}$
ADT	2.0 (1.1,3.5)	0.02
EBRT Only*	0.2 (0.04,0.9)	0.03
Implant + EBRT*	1.8 (1.0,3.1)	0.05

C.

Variable	*OR (95%CI)*	*p-value*
Cumulative SNP score	2.3 (1.9,2.8)	$2.2x10^{-19}$
Age	1.2 (1.1,1.2)	$2.3x10^{-13}$
ADT	2.9 (1.7,5.0)	$1.1x10^{-4}$
BED (10Gy2)	1.1 (0.9,1.2)	0.29
Prostate Volume ($10mm^3$)	1.1 (0.9,1.2)	0.09

Figure 2. ROC curves from multivariable logistic regression models. Clinical-only model contains age, ADT, and treatment; SNP-only model contains 12 SNPs and PCs; Clinical+SNP model contains age, ADT, treatment, 12 SNPs and PCs.



	AUC
Clinical-only	.75
SNPs-only	.79
Clinical + SNPs	.89

Table 4. A. Rank-correlation of cumulative SNP score and ED. SNPs with an additive inheritance pattern were counted as 0, 1, or 2 risk alleles; SNPs with a dominant or recessive inheritance pattern were counted as either 0 or 1 risk alleles with the appropriate genotypes collapsed into a single group. Sensitivity and specificity were calculated using predicted outcomes from the model including SNP score, age, ADT, treatment and PCs. Each row shows the sensitivity and specificity achieved if the SNP score was dichotomized at the number shown for that row. B. Relationship with cumulative SNP score and age. Percentages are based on row totals.

A.

Cumulative SNP score	***Cases, N(%)***	***Controls, N(%)***	***Sensitivity***	***Specificity***
3	0	2 (1.0%)	77.6%	58.5%
4	0	4 (2.1%)	77.6%	60.1%
5	1 (0.4%)	14 (7.3%)	79.3%	64.2%
6	4 (1.6%)	28 (14.5%)	82.1%	65.8%
7	17 (6.9%)	37 (19.2%)	84.6%	72.0%
8	38 (15.4%)	38 (19.7%)	83.3%	71.0%
9	48 (19.5%)	27 (14.0%)	80.9%	73.1%
10	56 (22.8%)	23 (11.9%)	78.5%	71.5%
11	45 (18.3%)	15 (7.8%)	77.2%	66.3%
12	21 (8.5%)	4 (2.1%)	77.2%	62.7%
13	12 (4.9%)	1 (0.5%)	77.6%	59.6%
14	3 (1.2%)	0	77.6%	59.1%
15	1 (0.4%)	0	76.2%	60.5%
Total	246	193	83.7%	74.6%

B.

	Case, N(%)	***Control, N(%)***	***Total***
≤ 62 years			
3-9 risk alleles	22 (21.8%)	79 (78.2%)	101
10-15 risk alleles	39 (52.7%)	35 (47.3%)	74
> 62 years			
3-9 risk alleles	86 (54.8%)	71 (45.2%)	157
10-15 risk alleles	99 (92.5%)	8 (7.5%)	107

Table S1. Multivariable ordered logistic regression of cumulative SNP score on post-treatment SHIM score category (includes individuals from the GWAS and individuals whose SHIM scores fell in the intermediate range; N=536). Post-treatment SHIM was categorized into 5 groups (1-7, 8-11, 12-16, 17-21, and 22-25) as previously published[10,15].

Variable	***OR (95%CI)***	***p-value***
Cumulative SNP score	1.5 (1.4,1.7)	$1.4x10^{-19}$
Age	1.1 (1.1,1.1)	$2.0x10^{-16}$
ADT	1.3 (0.9,1.9)	0.13
EBRT Only*	1.2 (0.2,6.1)	0.85
Implant + EBRT*	1.9 (1.3,2.8)	$6.7x10^{-04}$

Table S2. Association results between the top 31 SNPs identified in a published GWAS and ED outcome in the Mount Sinai cohort. The published GWAS was carried out in a small cohort of African American (AA) men (27 cases and 52 controls; p-values reported in the column labeled "p-value in AA GWAS"). The p-values and odds ratios (OR) reported in the right-hand side of the table are for association tests carried out in the discovery cohort of the current study (132 cases, 103 controls) using logistic regression controlling for age, ADT, RT type and ancestry. * the exact SNP from the AA GWAS was filtered out of the Mount Sinai dataset due to low minor-allele frequency; the reported SNP is the next nearest in distance to the excluded SNP.

p-value in AA GWAS	*dbSNPrsID*	*Nearest Gene*	*Chr*	*BP*	*A1*	*OR (95% CI) in Mount Sinai GWAS*	*p-value in Mount Sinai GWAS*	*Inheritance Model*
$5.5x10^{-08}$	rs2268363	FSHR	2	49,054,832	G	1.3 (0.7,2.58)	0.39	dominant
$4.7x10^{-07}$	rs10194115	TTC7A	2	47,093,516	T	1.5 (0.7,3.30)	0.34	additive
$5.9x10^{-07}$	rs2806864	PTGFRN	1	117,271,304	G	0.7 (0.3,1. 7)	0.38	dominant
$6.9x10^{-07}$	rs7064929	KIAA1166	23	64,283,744	A	3.3 (0.7,15.2)	0.12	additive
$8.3x10^{-07}$	rs10861905	CMKLR1	12	107,291,463	A	1.6 (0.7,3.6)	0.28	additive
$1.4x10^{-06}$	rs1527243	TSN	2	123,007,492	C	1.5 (0.7,3.0)	0.28	dominant
$1.5x10^{-06}$	**rs831270***	**CHMP5**	**9**	**33,262,424**	**A**	**1.9 (1.0,3.7)**	**0.05**	**dominant**
$2.0x10^{-06}$	rs2716734	TMEM178	2	39,801,225	T	0.8 (0.3,2.1)	0.72	dominant
$2.0x10^{-06}$	rs10210358	LRP1B	2	141,512,090	A	1.2 (0.8,2.0)	0.41	additive
$2.1x10^{-06}$	rs4920403*	IGSF21	1	18,166,435	G	1.5 (0.4,5.5)	0.56	recessive
$3.1x10^{-06}$	rs5926140*	DDX53	23	22,801,029	A	0.7 (0.3,1.7)	0.46	additive
$3.5x10^{-06}$	**rs7552382**	**PTGFRN**	**1**	**117,324,524**	**G**	**0.6 (0.4,0.9)**	**0.02**	**additive**
$3.6x10^{-06}$	**rs6741148**	**FAM82A1**	**2**	**38,131,336**	**G**	**0.3 (0.1,0.7)**	**$4.8x10^{-3}$**	**additive**
$3.8x10^{-06}$	rs3802458	C9orf3	9	96,781,095	G	1.7 (0.6,4.3)	0.28	additive
$4.3x10^{-06}$	rs6862844	ZNF608	5	124,393,866	C	0.4 (0.1,1.0)	0.06	dominant
$4.6x10^{-06}$	rs2901964	ELA2A	1	15,665,013	G	0.8 (0.4,1.6)	0.58	dominant
$4.8x10^{-06}$	rs11122834	GLI2	2	121,417,759	T	4.9 (0.4,67.3)	0.23	recessive
$5.7x10^{-06}$	rs9948	CNNM3	2	96,864,527	C	1.0 (0.5,1.9)	0.92	dominant
$6.3x10^{-06}$	rs5965182	HEPH	23	65,523,418	T	1.1 (0.5,2.5)	0.81	additive
$6.7x10^{-06}$	rs17005499	GLI2	2	121,425,911	A	1.2 (0.4,3.5)	0.70	additive
$7.2x10^{-06}$	rs943371	PTGFRN	1	117269213	C	0.7 (0.3,1.7)	0.38	additive
$7.3x10^{-06}$	rs5944185	MAGEB18	23	25,763,535	C	0.9 (0.4,2.0)	0.88	additive
$7.3x10^{-06}$	rs219553	APOB	2	21,431,248	A	0.7 (0.2,2.0)	0.51	recessive
$7.7x10^{-06}$	**rs6049375**	**GGTLC1**	**20**	**24,006,407**	**T**	**0.1 (0.02,0.5)**	**7.14E-3**	**recessive**
$7.7x10^{-06}$	rs5971305	WDR42B	23	28,142,974	G	0.9 (0.4,1.7)	0.67	additive
$8.9x10^{-06}$	rs2806863	PTGFRN	1	117,271,036	G	0.7 (0.3,1.7)	0.38	dominant
$9.3x10^{-06}$	rs743150	SYTL5	23	37,739,633	T	0.9 (0.5,1.6)	0.69	additive
$9.8x10^{-06}$	rs13408245	MKI67IP	2	122,183,723	T	1.61 (0.4,7.0)	0.53	additive
$9.9x10^{-06}$	**rs6432484**	**FAM84A**	**2**	**14,944,498**	**T**	**2.2 (1.1,4.5)**	**0.03**	**dominant**
$7.1x10^{-05}$	**rs17070658**	**CSMD1**	**8**	**4,425,687**	**T**	**2.1 (1.1,4.1)**	**0.04**	**dominant**
$7.1x10^{-05}$	**rs17070660**	**CSMD1**	**8**	**4,425,951**	**A**	**2.1 (1.1,4.1)**	**0.04**	**dominant**

Table S3. Association results for SNPs within genomic regions of top hits from African American GWAS. Regions were selected in the contained at least one SNP with p-value from the MSSM cohort $<1x10^{-2}$. All other SNPs with p-value <0.05 within that region are reported. SNPs in bold are the sentinel SNPs from the GWAS carried out in the African American cohort. The majority of the SNPs were genotyped in the discovery cohort for the current study (N=235). SNPs denoted with † were genotyped in both the discovery and replication cohorts (N=465). For "Distance from AA SNP", SNPs with a positive sign are located 3' of the sentinel SNP from the African American GWAS; SNPs with a negative sign are located 5' of the sentinel SNP from the African American GWAS.

Nearest Gene	dbSNPrsID	Distance (kb) from AA SNP	Chr	BP	A1	OR	L95	U95	P-value	Inheritance Model
	rs2268363	**0**		**49,054,832**	**G**	**1.2**	**0.7**	**2.1**	**0.5306**	
	rs1882558	46		49,101,241	G	0.6	0.4	1.0	0.0527	
FSHR	rs10495964	141	2	49,195,505	T	0.5	0.3	1.0	0.0341	Additive
	rs10495965	141		49,196,308	A	0.5	0.3	0.8	0.0086	
	rs17038285	142		49,196,505	A	0.5	0.3	1.0	0.0368	
	rs943371	**0**		**117,269,213**	**C**	**0.7**	**0.3**	**1.7**	**0.3843**	
	rs2806863	**0**		**117,271,036**	**G**	**0.7**	**0.3**	**1.7**	**0.3843**	
	rs2806864	**0**		**117,271,304**	**G**	**0.7**	**0.3**	**1.7**	**0.3843**	
	rs1998922	6		117,277,238	A	0.5	0.3	1.0	0.0397	
	rs6692981	10		117,281,275	T	0.3	0.1	0.6	0.0015	
	rs4641299	14		117,284,884	G	0.5	0.3	0.9	0.0283	
PTGFRN	rs12566708	-23	1	117,301,171	G	0.4	0.2	0.8	0.0134	Dominant
	rs12023283	-22		117,302,933	A	0.4	0.2	0.8	0.0125	
	rs10923182	-18		117,306,835	T	0.5	0.2	0.9	0.0149	
	rs10923183†	-16		117,308,255	G	0.6	0.4	0.9	0.0232	
	rs10923184†	-16		117,308,744	G	0.6	0.4	0.9	0.0263	
	rs4131408†	-16		117,308,925	C	0.6	0.4	1.0	0.0316	
	rs12090536	-8		117,316,638	G	0.5	0.3	0.9	0.0247	
	rs7552382	**0**		**117,324,524**	**G**	**NA**	**NA**	**NA**	**NA**	
	rs6049370	-12		23994836	G	2.2	1.1	4.3	0.0244	
	rs6049375	**0**		**24006407**	**T**	**1.3**	**0.7**	**2.5**	**0.4466**	
	rs1573008	3		24009186	A	0.5	0.3	0.8	0.0027	
	rs6049376	4		24010085	T	2.3	1.2	4.5	0.0145	
	rs6036634	4		24010198	A	2.1	1.1	4.0	0.0318	
	rs6049385	16		24021964	C	2.2	1.2	4.0	0.0101	
GGTLC1	rs6138201	16	20	24022349	T	0.4	0.3	0.7	0.0014	Additive
	rs6036641	20		24026476	G	2.6	1.2	5.5	0.0133	
	rs6036659	40		24046673	A	3.4	1.1	10.7	0.0372	
	rs6138211	42		24048323	C	2.8	1.3	6.2	0.0103	
	rs11699743†	56		24062150	A	1.5	1.1	2.0	0.0110	
	rs6138213	56		24062903	T	2.6	1.1	5.7	0.0234	
	rs7270353	67		24073147	A	0.5	0.3	0.8	0.0080	

	rs6049433	69		24075023	A	0.6	0.4	0.9	0.0254	
	rs6138224	77		24082917	A	2.6	1.2	5.3	0.0112	
	rs6049472	100		24106051	G	2.5	1.2	5.3	0.0171	
	rs8116594	101		24107081	G	0.5	0.3	0.9	0.0159	
	rs8125641	102		24108549	A	0.5	0.3	0.8	0.0084	
	rs8123478	105		24110977	C	0.6	0.4	0.9	0.0173	
	rs2749422	119		24125093	G	2.2	1.1	4.5	0.0324	
	rs7268880	125		24130992	A	0.6	0.4	0.9	0.0248	
	rs7272205	129		24135784	C	0.6	0.4	0.9	0.0167	
	rs565833	132		24138620	A	1.7	1.1	2.7	0.0228	
	rs562594	135		24141691	G	1.8	1.1	2.9	0.0152	
	rs504964	137		24143397	G	1.8	1.1	2.9	0.0130	
	rs502250	137		24143694	G	1.9	1.1	3.1	0.0165	
	rs501262	137		24143821	G	1.6	1.0	2.5	0.0334	
	rs573964	138		24144369	T	1.8	1.1	2.9	0.0134	
	rs473253	138		24144578	C	1.8	1.1	2.8	0.0182	
	rs478843	144		24150539	G	1.7	1.1	2.7	0.0268	
	rs6750019	-73		14871034	A	0.6	0.4	1.0	0.0465	
	rs10174079	-54		14890973	C	0.6	0.3	0.9	0.0283	
	rs2679421	-42		14902530	C	2.5	1.2	5.1	0.0156	
	rs4670032	-2		14942545	A	0.6	0.3	0.9	0.0281	
	rs6432484	**0**		**14944498**	**T**	**1.9**	**1.1**	**3.6**	**0.0357**	
	rs4670039	17		14961251	A	0.5	0.2	1.0	0.0454	
	rs7577131†	32		14976349	G	0.7	0.5	0.9	0.0103	
	rs17367970†	32		14976634	G	0.6	0.4	1.0	0.0312	
FAM84A	rs11885902	35	2	14979318	C	0.4	0.2	0.7	0.0014	Additive
	rs7594846†	36		14980349	A	0.7	0.5	0.9	0.0169	
	rs12478662	37		14981797	T	0.4	0.3	0.7	0.0017	
	rs4668842	44		14988606	A	0.4	0.2	0.8	0.0038	
	rs4670045	53		14997123	T	0.5	0.3	0.9	0.0116	
	rs793824	54		14998569	T	1.8	1.1	2.9	0.0204	
	rs12470390	55		14999712	C	0.5	0.3	0.8	0.0071	
	rs7568703	64		15008255	T	1.7	1.1	2.8	0.0240	
	rs4670049	81		15025247	C	0.6	0.3	1.0	0.0418	
	rs17415128	-116		4310065	G	2.8	1.1	7.0	0.0297	
	rs1217543	-83		4342751	G	0.6	0.4	1.0	0.0397	
CSMD1	rs6558891	-28	8	4398160	G	2.0	1.2	3.2	0.0044	Additive
	rs17070658	**0**		**4425687**	**T**	**2.0**	**1.0**	**3.9**	**0.0435**	
	rs17070660	**0**		**4425951**	**A**	0.5	0.3	0.9	0.0159	
	rs13269869	24		4450007	C	0.5	0.3	0.8	0.0084	

A Two-Stage Genome-Wide Association Study to Identify Single Nucleotide Polymorphisms Associated with Change in American Urological Association Symptom Score Following Radiotherapy for Prostate Cancer

This research was supported by grants RSGT-05-200-01-CCE from the American Cancer Society, PC074201 from the Department of Defense and 1R01CA134444 from the National Institutes of Health.

Introduction

Late urinary morbidity is a common adverse effect of radiation therapy for prostate cancer. Late effects include bothersome symptoms that affect quality of life, such as increased urinary frequency, incomplete bladder emptying, weak urinary stream and incontinence, as well as more serious events such as bladder necrosis or hemorrhagic cystitis. Whereas risk factors for acute urinary morbidity have been well-documented, acute urinary morbidity does not predict strongly for late urinary effects, and few risk factors for late effects have been identified.[1] A study from 2003 comparing brachytherapy patients to a group of newly diagnosed (and not yet treated) control patients found that tobacco use was predictive of long term urinary symptoms, but did not find a significant association with other factors investigated including tumor stage, Gleason score, patient age, hormone use and prostate volume.[2] A larger study of clinical predictors of late urinary symptoms found that pre-treatment American Urological Association Symptom Score (AUASS), prostate volume and use of adjuvant hormone therapy were significantly associated with change in AUASS during the first 1 to 3 years following permanent seed implantation but not during later follow-up intervals.[3]

It has been hypothesized that genetic factors may predispose individuals to development of late adverse effects following exposure to radiation therapy. Several candidate gene studies have been conducted to identify single nucleotide polymorphisms (SNPs) that are associated with late urinary symptoms. In one study among a small (N = 83) group of prostate cancer patients treated with three-dimensional conformal radiotherapy, SNPs in *MLH1*, *XRCC3*, *LIG4*, and *CYP2D6*4* were significantly associated with combined bladder and/or rectal toxicity.[4] Similarly, another small study (N = 41) found SNPs in *LIG4* and *MDC1* to be associated with combined late GU/GI toxicity.[5] In another study, a multi-SNP model including SNPs in *ART1*, *ID3*, *EPDR1*, *PAH*, and *XRCC6*, was found to be predictive of radiation cystitis among a group of men (n = 197) treated with carbon ion radiotherapy.[6] Few, if any, candidate gene SNP associations have been replicated in follow-up studies.

The genetic association studies to date have been conducted using relatively small sample sizes, and many look at single measures of urinary toxicity (ex. cystitis) or combined GU and GI toxicity as endpoints. Few studies account for baseline symptoms. To the best of our knowledge, no genetic association studies have been conducted with change in overall urinary morbidity, relative to pre-RT symptom levels, as an endpoint. Furthermore, few of the studies have investigated genetic factors affecting pathways outside of DNA damage and radiation response. Using a more broad approach, we undertook a two-stage genome-wide association study (GWAS) among a group of 723 men treated with radiotherapy for prostate cancer with change in AUASS at several time periods following RT as the primary endpoint.

Materials and Methods

Patient Characteristics

Men treated with radiation therapy for biopsy proven adenocarcinoma of the prostate were recruited from the Mount Sinai Medical Center, following informed consent. This study was approved by the Mount Sinai Medical Center Institutional Review Board.

Radiation therapy consisted of brachytherapy alone with a full ^{125}Iodine implant prescribed to 160Gy (TG43) or a partial ^{103}Palladium implant prescribed to 100Gy followed in 6–8 weeks by external beam irradiation (3D conformal or image guided intensity modulated radiation therapy, IMRT) to 24 to 50Gy (median 45Gy). All implants were performed by or under the direct supervision of a single physician (RS). Delivered radiation doses were converted to the biologically effective dose (BED) using an α/β of 2Gy, as described previously.[7] The patients were stratified into low-risk, medium-risk and high-risk disease recurrent groups as previously described.[8] Hormone therapy was administered for 3-6 months in low-risk patients with prostate volumes >50cc, in intermediate-risk patients for 6 months with brachytherapy monotherapy or for 9-24 months in high-risk patients with combination brachytherapy and EBRT.

Patients were assessed prior to RT (baseline) and followed prospectively approximately every 6 months with the AUASS questionnaire[9]. The primary endpoint of the study was change in AUASS relative to baseline at each of 4 follow-up periods (1-2yrs, 2-3yrs, 3-4yrs and 4-5yrs). If a patient had two AUAS scores reported during a one-year time period, the later of the two scores was considered. All patients with pre-RT AUASS available, high-quality DNA and at least 1-year of follow-up (N = 723) were included. The 723 patients who met the inclusion criteria were randomly split into a discovery cohort (N = 346) and a replication cohort (N=377). The two groups were similar with respect to patient demographics, clinical variables and length of follow-up with AUASS questionnaire (Table 1).

Genotyping

Genomic DNA was isolated from lymphocytes as previously described.[10] DNA samples from the discovery cohort were genotyped for ~909,000 SNPs using Affymetrix v6.0 arrays (Affymetrix, Santa Clara, CA), and genotypes were called using Genotyping Console. Top SNPs were selected from the discovery analysis, and DNA from the replication cohort was genotyped for these SNPs using Illumina iSelect custom arrays (Illumina, San Diego, CA). Genotypes from the Illumina arrays were called by GenomeStudio. SNPs were excluded from analysis if missing in >5% of samples (137,589 SNPs in the discovery dataset; 22 SNPs in the replication dataset) or if they had minor allele frequency <5% (157,580 SNPs in the discovery dataset) or if they showed deviation from Hardy-Weinberg equilibrium (p-value < $1x10^{-5}$; 957 SNPs in the discovery dataset). Individuals were excluded if they showed cryptic relatedness as assessed by pairwise identity-by-state (8 pairs) or if they had call rate <90% (N=2). Duplicate control samples included in both rounds of genotyping showed >95% concordance. Three control samples (a trio of two parents and an offspring) were included on both the Affymetrix and Illumina genotyping platforms and showed >99% concordance across platforms. After quality-control filtering, the discovery dataset included 613,496 SNPs, of which 1,374 with discovery cohort p-value <0.01 at multiple follow-up periods were selected for testing in the replication cohort. PLINK was used to perform QC and association tests.[11]

Principal components analysis (PCA) was performed to obtain estimates of genetic ancestry. PCA was carried out using 860 ancestry-informative SNPs among the study subjects and 11 reference populations from the International HapMap Project.[12] PCs 1 through 5 were included in genetic association tests to adjust for individual ancestry. Inclusion of PCs appeared to adequately control for ancestry as evidenced by a low genomic inflation factor of 1.00 after adjustment. SNP data processing and PCA was carried out using R 2.13.1.[14]

Statistical Analysis

Association between non-genetic variables and change in AUASS was assessed by univariate and multivariate linear regression, and significance was assessed by t test for continuous variables and Wald chi-square for categorical variables. Association between SNPs and change in AUASS was similarly assessed by multivariate linear regression adjusting for the first five principal components from PCA. Combined p-values for the discovery and replication cohorts were calculated using Fisher's trend method after filtering for agreement in

beta coefficient direction and inheritance model (out of additive, dominant and recessive models). IBM SPSS Statistics 19 was used for univariate and multivariate analysis. Haploview v4.2 was used to obtain linkage disequilibrium values and heatmap based on the combined HapMap CEU and TSI populations.[13]

Results

A total of 723 men treated with definitive radiotherapy (RT) for prostate cancer were included in the study. Men were followed up for a mean of 46.8 months (s.d. 13.6 months). The mean and median AUASS at baseline and at each time period following treatment are shown in Figure 1. On average, the highest AUAS scores were seen during the 1-2 year and 2-3 year time periods (mean AUASS at 1-2 years is 12.3 and at 2-3 years is 10.4 compared to 7.5 at baseline), with most men returning to baseline score by 5 years post-RT. The mean change in AUASS relative to baseline is shown by pre-RT severity group in Figure 2.

We carried out a two-stage genome-wide association study to identify SNPs associated with change in AUASS at one or more of the time periods following RT. Because there was wide variation in pre-RT AUASS among the patients included in the study, we adjusted for pre-RT AUASS severity group in the genetic association tests. We also adjusted for PCs 1 through 5 to control for individual ancestry. A total of 33 SNPs tagging 18 genomic regions were identified in the discovery cohort and validated in the replication cohort (Supplementary Table 1). A region on chromosome 9p21.2 tagged by 8 SNPs showed the strongest association with change in AUASS in both the discovery and replication cohorts, with a combined p-value suggestive of genome-wide significance (Table 2). Out of the group, SNP rs10967965 showed the strongest association signal with a beta coefficient of 2.7 (95% CI 1.2, 4.1) in the discovery cohort and 2.4 (95% CI 1.1, 3.6) in the replication cohort (discovery cohort p-value = 3.7×10^{-4}, replication cohort p-value = 1.9×10^{-4}; combined p-value = 6.6×10^{-7}) at the 2-3yr time period. Individuals with the risk allele for rs10967965 experienced, on average, a 4.7 point increase in AUASS during the 2-3 year follow-up period whereas individuals without the risk allele for rs10967965 experienced, on average, a 2.6 point increase in AUASS during the 2-3 year follow-up period. For all 8 SNPs in this region, a positive association with change in AUASS is seen across all time periods, though the magnitude of effect and statistical significance is greatest during the 2-3 year time period. Additional support for this association is given by the finding that these SNPs reside in a haplotype block, which itself is associated with change in AUASS (Figure 3). In addition to the region on 9p21.2, we identified 24 SNPs tagging 10 genomic loci that showed moderate significance for association with change in AUASS at multiple follow-up periods across both the discovery and replication cohorts (supplementary table 1).

We analyzed clinical and treatment variables to determine whether any are associated with change in AUASS, and to see if the SNPs on 9p21.2 are independently significant after adjusting for non-genetic factors. On univariate analysis, hypertension at time of diagnosis ($p = 0.026$ at 1-2yrs post-RT), use of alpha-blockers following RT ($p = 0.006$ at 1-2yrs post-RT), and smoking history ($p = 0.045$ at 1-2yrs post-RT; $p = 0.048$ at 2-3yrs post-RT) are significantly associated with a higher AUASS following RT (Table 3A). Conversely, concomitant hormone therapy ($p = 0.012$ at 1-2yrs post-RT; $p = 0.040$ at 2-3yrs post-RT), age at time of diagnosis ($p = 0.024$ at 1-2yrs post-RT), and pre-RT AUASS ($p < 0.001$ at all follow-up time periods) are significantly associated with a lower AUASS following RT (Table 3A). While hormone therapy, alpha-blocker use, hypertension, smoking history and age were only significantly associated with change in AUASS at the earlier follow-up period(s), pre-RT AUASS was strongly associated with change in AUASS at all follow-up periods. The inverse association between age and change in AUASS following treatment may be due, in part, to confounding by pre-RT AUASS. Indeed, when pre-RT AUASS severity category is adjusted for, the association between age and change in AUASS no longer reaches statistical significance (Beta coefficient = -0.3, 95% CI -0.6,0.1; $p = 0.101$).

When all variables that were significant on univariate analysis are combined in multivariate regression, smoking history, hypertension at time of diagnosis, use of alpha-blockers following RT and pre-RT AUASS remain independently associated with change in AUASS (Table 3B). When rs10967965 is added into the multivariate regression model, it is independently associated with change in AUASS (beta coefficient = 2.5; 95% CI 1.5, 3.5; p-value = 2.9×10^{-6}) at the 2-3yr time period.

To better understand the biology behind the SNP associations indentified in this GWAS, we looked to see whether the SNPs associated with change in *total* AUASS were more strongly associated with certain *individual* items on the AUASS questionnaire compared to others. While many of the SNPs appeared to be equally associated with each individual AUASS item, several showed a particularly strong association with one or more specific items (supplementary table 2). In particular, the SNPs on 9p21.2 were most strongly associated with increase in score for the "incomplete emptying", "intermittency" and "frequency" questions. Interestingly, SNP rs13035033 on chromosome 2q31.1 showed only a moderate association with overall change in AUASS (supplementary table 1), but a very strong association with the AUASS question on "straining" to urinate (beta-coefficient 0.9, 95%CI 0.6,1.2; p-value 5.0×10^{-9}). This SNP lies within the *MYO3B* gene which encodes the actin-based motor protein myosin IIIB and is highly expressed in the kidney.[14]

Discussion

The cluster of SNPs on 9q21.2, identified by this genome-wide association study, reside within, upstream and downstream of the interferon kappa (*IFNK*) gene and within the MOB kinase activator 3B (*MOB3B*; formerly *MOBKL2B*) gene, both of which share a genomic locus and are transcribed from opposite strands of the DNA. The cluster forms a 44kb haplotype block that is centered on a region encompassing the entire *IFNK* gene. The hapotype itself is associated with increase in urinary symptoms following RT, and while the SNPs identified in this study are likely not causal themselves, they appear to tag a block that may contain a causal, protein-coding variant. Fine-mapping studies will be needed to identify such a variant. Interestingly, the genes residing at this locus have biological functions that suggest a role in urinary morbidity following radiation. The yeast homolog of *MOB3B* is binding partners with a protein kinase essential for spindle pole body duplication and mitotic checkpoint regulation.[15] *IFNK* is a member of the type I interferon family of immune system genes and has been shown to directly module cytokine release by cells of the innate immune system, inhibiting IL-12 signaling in particular.[16] Urinary symptoms following RT are thought to be a manifestation of inflammatory response to the tissue damage by radiation.[17] In a mouse model, IL-12 was shown to limit migration of MHC class II immune cells following exposure to ionizing radiation.[18] It is thus biologically plausible that the *IFNK* gene could play a role in modulating this type of inflammatory response in the tissues affecting urinary symptoms.

The finding that the risk genotype for the rs10967965 SNP was associated with an average 4.7 point increase in AUASS over baseline is a clinically relevant finding. Previous work has shown that a 5 point increase in AUASS from baseline was found previously to be associated with a worse score for the quality of life question on the International Prostate Symptom Score (IPSS), an expanded version of the AUASS.[19] Though the effect size will likely be refined through further validation studies, the point estimate obtained here suggests that it is at least in the range of clinical utility in terms of predicting the effect on a given patient's experience following treatment.

Use of change in AUASS from baseline score has, to the best of our knowledge, not been used as a measure of urinary morbidity in response to radiation therapy in previous genetic association studies. We think this approach is superior to using a cut-off of an absolute AUASS value at a certain threshold, because this approach would select for individuals who had elevated baseline scores rather than those men who are experiencing a late

radiation effect. Also, unlike normal tissue adverse outcomes such as erectile dysfunction or rectal bleeding, the distribution of urinary symptom scores closely approximate a normal curve rather than bimodal distribution which would lend itself to dichotomization into a case/control outcome. We believe we've captured this outcome most accurately by treating it as a quantitative trait.

Conclusions

This study represents the first genome-wide association study to date investigating genetic variants associated with urinary morbidity following radiation therapy for prostate cancer. A biologically plausible locus was identified containing a haplytype block which encompasses the inflammatory signaling protein interferon kappa. SNPs tagging this locus were independently associated with change in AUA symptom score after controlling for clinical and treatment factors associated with urinary symptoms. Validation in additional cohorts will be needed to confirm this association and to identify additional variants with more modest effect sizes.

References

1. Bittner N, Merrick GS, Wallner KE, Lief JH, Butler WM, Galbreath RW. The impact of acute urinary morbidity on late urinary function after permanent prostate brachytherapy. Brachytherapy. 2007 Oct-Dec;6(4):258-66.
2. Merrick GS, Butler WM, Wallner KE, Galbreath RW, Lief JH. Long-term urinary quality of life after permanent prostate brachytherapy. International journal of radiation oncology, biology, physics. 2003 Jun 1;56(2):454-61.
3. Stone NN, Gerber NK, Blacksburg S, Stone J, Stock RG. Factors influencing urinary symptoms 10 years after permanent prostate seed implantation. J Urol. 2012 Jan;187(1):117-23.
4. Damaraju S, Murray D, Dufour J, Carandang D, Myrehaug S, Fallone G, et al. Association of DNA repair and steroid metabolism gene polymorphisms with clinical late toxicity in patients treated with conformal radiotherapy for prostate cancer. Clin Cancer Res. 2006 Apr 15;12(8):2545-54.
5. Pugh TJ, Keyes M, Barclay L, Delaney A, Krzywinski M, Thomas D, et al. Sequence variant discovery in DNA repair genes from radiosensitive and radiotolerant prostate brachytherapy patients. Clin Cancer Res. 2009 Aug 1;15(15):5008-16.
6. Suga T, Iwakawa M, Tsuji H, Ishikawa H, Oda E, Noda S, et al. Influence of multiple genetic polymorphisms on genitourinary morbidity after carbon ion radiotherapy for prostate cancer. International journal of radiation oncology, biology, physics. 2008 Nov 1;72(3):808-13.
7. Stock RG, Stone NN, Cesaretti JA, Rosenstein BS. Biologically effective dose values for prostate brachytherapy: effects on PSA failure and posttreatment biopsy results. International journal of radiation oncology, biology, physics. 2006 Feb 1;64(2):527-33.
8. Stone NN, Stock RG. Prostate brachytherapy: treatment strategies. J Urol. 1999 Aug;162(2):421-6.
9. Barry MJ, Fowler FJ, Jr., O'Leary MP, Bruskewitz RC, Holtgrewe HL, Mebust WK, et al. The American Urological Association symptom index for benign prostatic hyperplasia. The Measurement Committee of the American Urological Association. J Urol. 1992 Nov;148(5):1549-57; discussion 64.
10. Atencio DP, Iannuzzi CM, Green S, Stock RG, Bernstein JL, Rosenstein BS. Screening breast cancer patients for ATM mutations and polymorphisms by using denaturing high-performance liquid chromatography. Environ Mol Mutagen. 2001;38(2-3):200-8.
11. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 2007 Sep;81(3):559-75.
12. Consortium IH. The International HapMap Project. Nature. 2003 Dec 18;426(6968):789-96.
13. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics. 2005 Jan 15;21(2):263-5.

14. Nambiar R, McConnell RE, Tyska MJ. Myosin motor function: the ins and outs of actin-based membrane protrusions. Cell Mol Life Sci. 2010 Apr;67(8):1239-54.
15. Chow A, Hao Y, Yang X. Molecular characterization of human homologs of yeast MOB1. Int J Cancer. 2010 May 1;126(9):2079-89.
16. Nardelli B, Zaritskaya L, Semenuk M, Cho YH, LaFleur DW, Shah D, et al. Regulatory effect of IFN-kappa, a novel type I IFN, on cytokine production by cells of the innate immune system. J Immunol. 2002 Nov 1;169(9):4822-30.
17. Robbins ME, Zhao W. Chronic oxidative stress and radiation-induced late normal tissue injury: a review. Int J Radiat Biol. 2004 Apr;80(4):251-9.
18. Cummings RJ, Mitra S, Foster TH, Lord EM. Migration of skin dendritic cells in response to ionizing radiation exposure. Radiat Res. 2009 Jun;171(6):687-97.
19. Cesaretti JA, Stone NN, Stock RG. Urinary symptom flare following I-125 prostate brachytherapy. International journal of radiation oncology, biology, physics. 2003 Jul 15;56(4):1085-92.

Table 1. Patient characteristics. Continuous variables are reported as mean (standard deviation). Categorical and dichotomous variables are reported as number (percent).

	Stage I Discovery Cohort (N = 346)	Stage II Replication Cohort (N = 377)	All Patients (N = 723)
Age (years)	64 (7)	65 (7)	65 (7)
Stage			
T1	188 (54.3%)	182 (48.3%)	373 (50.9%)
T2	145(41.9)	183 (48.5%)	334 (45.6%)
T3	13 (3.8%)	12 (3.2%)	26 (3.5%)
Gleason score			
≤ 6	222 (64.2%)	220 (58.4%)	446 (60.8%)
7	92 (26.6%)	108 (28.6%)	202 (27.6%)
≥ 8	32 (9.2%)	49 (13.0%)	85 (11.6%)
TURP prior to RT	5 (1.4%)	16 (4.2%)	22 (3.0%)
Initial PSA (ng/ml)	9.3 (17.9)	8.8 (9.7)	9.0 (14.2)
Androgen deprivation therapy	185 (53.5%)	199 (52.8%)	389 (53.1%)
Treatment			
Brachytherapy only	191 (55.2%)	215 (57.0%)	406 (55.4%)
Brachytherapy + EBRT	155 (44.8%)	162 (43.0%)	317 (43.2%)
Total BED (Gy2)	203.8 (21.6)	200.6 (26.5)	202.1 (24.3)
Prostate volume (cm^3)	45.7 (17.2)	47.9 (19.0)	46.9 (18.2)
Smoker	134 (38.7%)	173 (45.9%)	307 (41/9%)
Diabetes	19 (5.5%)	23 (6.1%)	43 (5.9%)
Hypertenstion	125 (36.1%)	122 (32.4%)	248 (33.8%)
Use of alpha-blocker following RT	186 (53.8%)	202 (53.6%)	394 (53.8%)
Follow-up (months)	47.9 (12.6)	45.7 (14.4)	46.8 (13.6)
Pre-RT AUASS			
0-7	212 (61.3%)	219 (58.1%)	433 (59.1%)
8-19	122 (35.3%0	132 (35.0%)	260 (35.5%)
20-35	12 (3.5%)	26 (6.9%)	40 (5.5%)

Figure 1. Distribution of AUASS at baseline (pre-RT) and each time period following RT from 1 to 5 years.

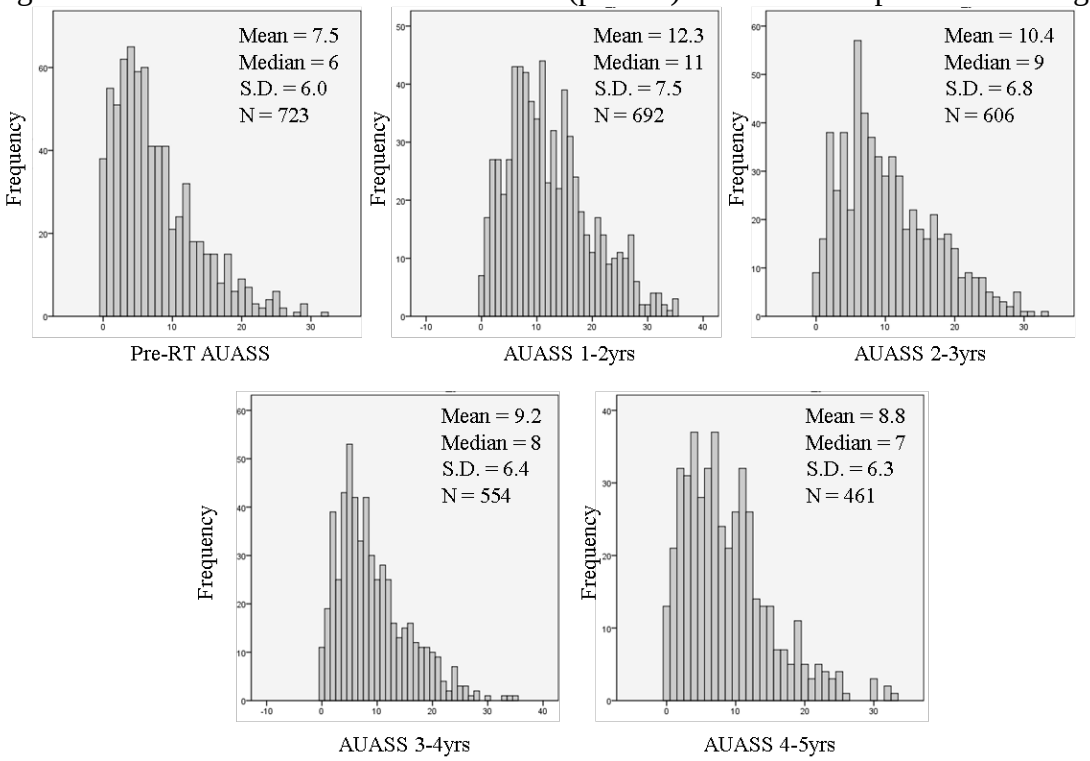


Figure 2. Mean change in AUASS following RT stratified by pre-RT score group.

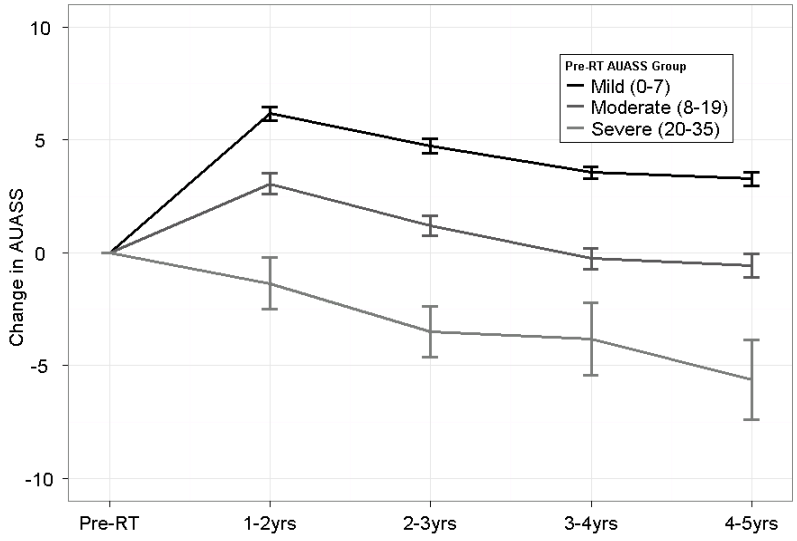


Table 2. SNPs on chromosome 9p21.2 associated with change in AUASS at following RT. Results are from linear regression adjusted for pre-RT AUASS and PCs1-5. Combined p-value was calculated using the Fisher trend method. BP position is for genome build 37/hg19.

					1-2yrs					2-3yrs				
					Discovery Cohort		Replication Cohort			Discovery Cohort		Replication Cohort		
dbSNPrsID	BP	Alleles	Discovery cohort MAF	Replication cohort MAF	Beta coefficient (95% CI)	p-value	Beta coefficient (95% CI)	p-value	combined p-value	Beta coefficient (95% CI)	p-value	Beta coefficient (95% CI)	p-value	combined p-value
rs17779457	27,488,342	C/A	0.24	0.25	2.0 (0.3,3.6)	$1.7x10^{-2}$	1.6 (0.3,2.9)	$1.6x10^{-2}$	$1.4X10^{-3}$	2.7 (1.2,4.1)	$3.7x10^{-4}$	2.4 (1.1,3.6)	$1.9x10^{-4}$	$6.6x10^{-7}$
rs10812604	27,496,911	A/C	0.33	0.34	2.6 (0.9,4.2)	$2.1x10^{-3}$	0.8 (-0.5,2.2)	0.225	$2.4x10^{-3}$	3.1 (1.6,4.6)	$4.2x10^{-5}$	1.7 (0.4,3.0)	$9.3x10^{-3}$	$3.4x10^{-6}$
rs10967965	27,498,238	T/A	0.17	0.18	1.8 (0.0,3.6)	$5.5x10^{-2}$	1.4 (0.0,2.8)	$5.7x10^{-2}$	$1.3x10^{-2}$	3.2 (1.6,4.8)	$8.6x10^{-5}$	2.0 (0.6,3.4)	$3.8x10^{-3}$	$2.8x10^{-6}$
rs1537712	27,508,739	A/G	0.29	0.30	2.1 (0.5,3.7)	$8.7x10^{-3}$	1.2 (-0.1,2.5)	$7.3x10^{-2}$	$3.1x10^{-3}$	2.3 (0.8,3.7)	$2.4x10^{-3}$	2.3 (1.1,3.5)	$2.6x10^{-4}$	$5.2x10^{-6}$
rs774354	27,516,217	G/A	0.29	0.30	2.1 (0.5,3.7)	$8.9x10^{-3}$	1.2 (0.0,2.5)	$5.9x10^{-2}$	$2.6x10^{-3}$	2.2 (0.8,3.6)	$2.5x10^{-3}$	2.3 (1.0,3.5)	$3.3x10^{-4}$	$6.8x10^{-6}$
rs774352	27,516,840	G/A	0.29	0.30	2.2 (0.6,3.8)	$7.6x10^{-3}$	1.2 (0.0,2.5)	$5.9x10^{-2}$	$2.3x10^{-3}$	2.2 (0.7,3.7)	$3.3x10^{-3}$	2.3 (1.0,3.5)	$3.3x10^{-4}$	$8.8x10^{-6}$
rs700782	27,526,297	T/C	0.25	0.25	1.9(0.3,3.5)	$1.7x10^{-2}$	1.3 (0.1,2.6)	$4.3x10^{-2}$	$3.5x10^{-3}$	2.6 (1.2,4.1)	$4.3x10^{-4}$	2.2 (1.0,3.5)	$4.4x10^{-4}$	$1.7x10^{-6}$
rs2453552	27,527,762	G/T	0.27	0.27	2.1 (0.6,3.7)	$8.2x10^{-3}$	1.3 (0.0,2.6)	$4.5x10^{-2}$	$1.9x10^{-3}$	2.6 (1.1,4.0)	$4.7x10^{-4}$	2.0 (0.8,3.3)	$1.5x10^{-3}$	$5.8x10^{-6}$

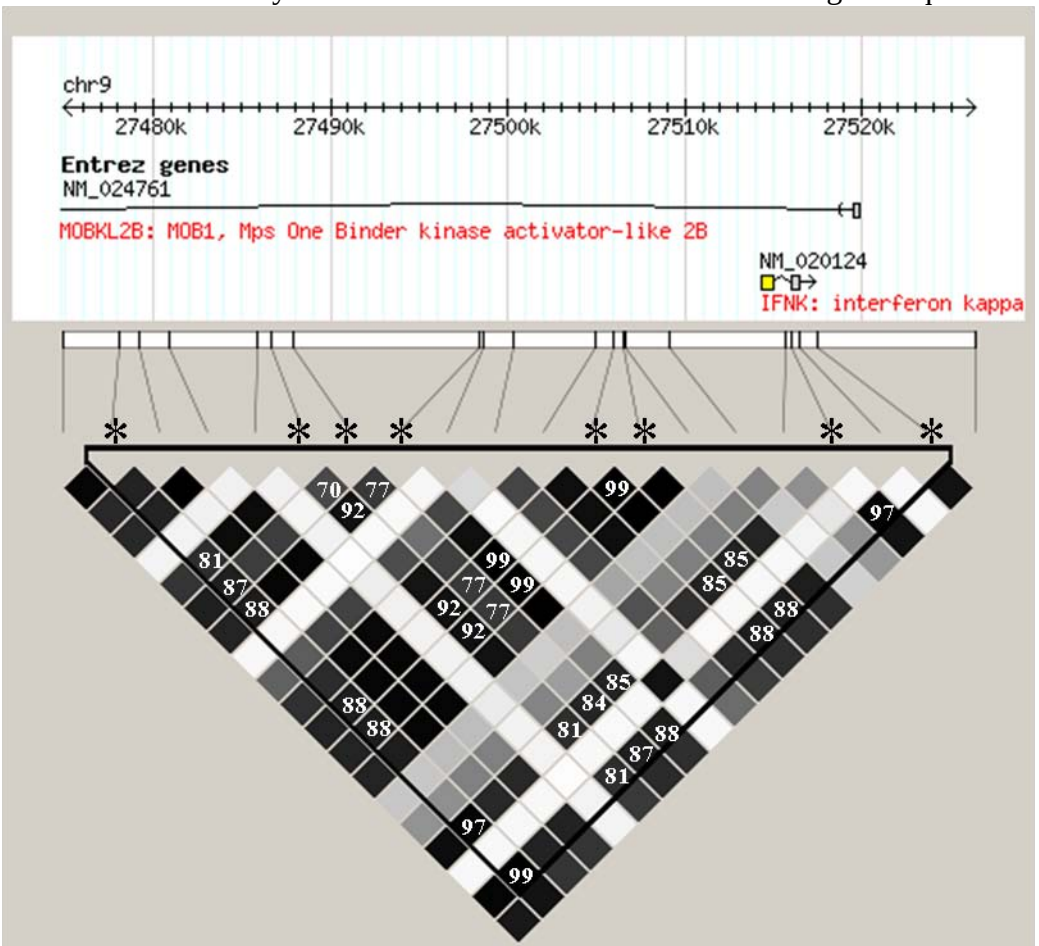
	3-4yrs					4-5yrs				
	Discovery Cohort		Replication Cohort			Discovery Cohort		Replication Cohort		
dbSNPrsID	Beta coefficient (95% CI)	p-value	Beta coefficient (95% CI)	p-value	combined p-value	Beta coefficient (95% CI)	p-value	Beta coefficient (95% CI)	p-value	combined p-value
rs17779457	2.6 (1.1,4.1)	$5.1x10^{-4}$	1.2 (-0.1,2.4)	$6.9x10^{-2}$	$2.0x10^{-4}$	2.1 (0.4,3.8)	$1.6x10^{-2}$	0.7 (-0.6,2.0)	0.300	$3.0x10^{-2}$
rs10812604	2.8 (1.3,4.3)	$2.3x10^{-4}$	0.7 (-0.6,2.0)	0.290	$4.0x10^{-4}$	2.2 (0.4,3.9)	$1.5x10^{-2}$	0.9 (-0.5,2.2)	0.209	$1.3x10^{-2}$
rs10967965	3.1 (1.5,4.7)	$1.2x10^{-4}$	1.5 (0.2,2.8)	$2.8x10^{-2}$	$2.5x10^{-5}$	2.8 (0.9,4.6)	$3.5x10^{-3}$	0.3 (-1.2,1.7)	0.728	$1.1x10^{-2}$
rs1537712	2.1 (0.6,3.5)	$5.9x10^{-3}$	1.1 (-0.1,2.3)	$8.3x10^{-2}$	$2.5x10^{-3}$	1.6 (-0.1,3.6)	$5.8x10^{-2}$	0.6 (-0.7,1.9)	0.353	$6.4x10^{-2}$
rs774354	2.0 (0.5,3.4)	$7.7x10^{-3}$	1.1 (-0.1,2.3)	$8.1x10^{-2}$	$3.0x10^{-4}$	1.6 (0.0,3.3)	$5.6x10^{-2}$	0.6 (-0.7,1.8)	0.399	$6.9x10^{-2}$
rs774352	1.9 (0.4,3.4)	$1.5x10^{-2}$	1.1 (-0.1,2.3)	$8.1x10^{-2}$	$5.5x10^{-3}$	1.3 (-0.5,3.0)	0.150	0.6 (-0.7,1.8)	0.399	0.156
rs700782	2.8 (1.3,4.2)	$2.0x10^{-4}$	0.9 (-0.3,2.1)	0.161	$2.0x10^{-4}$	1.8 (0.1,3.5)	$3.6x10^{-2}$	0.8 (-0.5,2.1)	0.206	$2.7x10^{-2}$
rs2453552	2.8 (1.4,4.3)	$1.4x10^{-4}$	0.8 (-0.4,2.0)	0.204	$1.8x10^{-4}$	1.7 (0.0,3.4)	$4.5x10^{-2}$	0.5 (-0.8,1.8)	0.424	$6.1x10^{-2}$

Supplementary Table 1. Top SNPs associated with change in AUASS at one or more follow-up period(s). Follow-up periods showing the strongest association for each SNP are shaded.

			1-2yrs post-RT					2-3yrs post-RT				
			Discovery Cohort		Replication Cohort			Discovery Cohort		Replication Cohort		
dbSNPrsID	Chr	Nearest Gene(s)	Beta (95%CI)	p-value	Beta (95%CI)	p-value	Combined p-value	Beta (95%CI)	p-value	Beta (95%CI)	p-value	Combined p-value
rs3818568	1p21.2	AGL	1.8 (0.3,3.2)	$1.6x10^{-2}$	1.9(0.7,3.1)	$2.1x10^{-3}$	$2.0x10^{-4}$	1.7 (0.4,3.1)	$1.3x10^{-2}$	1.4 (0.2,2.6)	$2.7x10^{-2}$	$1.8x10^{-3}$
rs13401638	2p21	PRKCE	3.9 (0.1,7.6)	$4.3x10^{-2}$	3.8 (1.1,6.5)	$5.4x10^{-3}$	$1.2x10^{-3}$	4.6 (1.2,8.0)	$8.3x10^{-3}$	1.4 (-1.2,4.0)	0.31	$1.1x10^{-2}$
rs13404973			3.9 (0.1,7.6)	$4.3x10^{-2}$	3.1 (0.5,5.7)	$1.9x10^{-2}$	$3.9x10^{-3}$	4.6 (1.2,8.0)	$8.3x10^{-3}$	0.8 (-1.7,3.4)	0.52	$1.7x10^{-2}$
rs13405086			3.9 (0.1,7.6)	$4.3x10^{-2}$	3.1 (0.5,5.7)	$1.9x10^{-2}$	$3.9x10^{-3}$	4.6 (1.2,8.0)	$8.3x10^{-3}$	0.8 (-1.7,3.4)	0.52	$1.7x10^{-2}$
rs4953253			4.1 (0.9,7.3)	$1.3x10^{-2}$	2.9 (0.5,5.2)	$1.6x10^{-2}$	$1.1x10^{-3}$	3.6 (0.5,6.7)	$2.4x10^{-2}$	0.9 (-1.4,3.3)	0.43	$3.6x10^{-2}$
rs13035033	2q31.1	MYO3B	4.9 (1.5,8.2)	$4.7x10^{-3}$	0.8 (-2.0,3.5)	0.58	$1.1x10^{-2}$	5.9 (2.9,8.9)	$1.4x10^{-4}$	3.3 (0.8,5.8)	$1.1x10^{-2}$	$1.2x10^{-5}$
rs9866974	3p24.1	RBMS3	3.1 (1.0,5.1)	$3.7x10^{-3}$	2.0 (0.0,4.0)	$5.6x10^{-2}$	$1.1x10^{-3}$	3.2 (1.3,5.0)	$7.9x10^{-4}$	1.9 (0.0,3.8)	$5.1x10^{-2}$	$3.0x10^{-4}$
rs6764017	3q13.13	PVRL3/FLJ25363	3.3 (1.1,5.4)	$2.8x10^{-3}$	2.3 (0.5,4.1)	$1.1x10^{-2}$	$2.0x10^{-4}$	1.4 (-0.5,3.4)	0.15	1.7 (0.0,3.5)	$5.7x10^{-2}$	$3.1x10^{-2}$
rs865808	3q27.1	EIF2B5	0.9 (-0.2,1.9)	0.120	0.8 (-0.1,1.6)	$9.4x10^{-2}$	$3.9x10^{-2}$	1.3 (0.3,2.3)	$9.2x10^{-3}$	0.3 (-0.6,1.1)	0.56	$2.0x10^{-2}$
rs7676349	4q22.2	GRID2	1.8 (-1.2,4.8)	0.23	1.8 (-0.6,4.2)	0.14	$9.4x10^{-2}$	3.3 (0.6,6.0)	$1.6x10^{-2}$	0.8 (-1.5,3.2)	0.49	$2.8x10^{-2}$
rs41385744			1.8 (-1.7,5.4)	0.31	0.2 (-2.6,3.1)	0.87	0.50	2.9 (-0.3,6.1)	$7.6x10^{-2}$	0.6 (-2.2,3.4)	0.67	0.14
rs16883632	6p12.1	GCM1/ELOVL5	2.1 (0.2,4.0)	$3.2x10^{-2}$	1.4 (-0.2,3.1)	$9.6x10^{-2}$	$1.3x10^{-2}$	1.4 (-0.3,3.2)	0.11	2.1 (0.5,3.7)	$1.2x10^{-2}$	$6.0x10^{-3}$
rs9390419	6q24.3	C6orf103	1.1 (-0.4,2.5)	0.15	0.7 (-0.6,1.9)	0.29	0.12	2.6 (1.2,3.9)	$1.9x10^{-4}$	1.3 (0.1,2.5)	$4.1x10^{-2}$	$5.5x10^{-5}$
rs9403813			1.3 (-0.2,2.7)	$9.4x10^{-2}$	0.6 (-0.7,1.8)	0.36	$9.8x10^{-2}$	2.4(1.1,3.8)	$5.4x10^{-4}$	1.4 (0.2,2.6)	$2.8x10^{-2}$	$1.0x10^{-4}$
rs6463707	7p21.3	MIOS/RPA3	-2.9 (-5.2,-0.6)	$1.5x10^{-2}$	-1.8 (-3.4,-0.2)	$2.9x10^{-2}$	$2.2x10^{-3}$	-2.4 (-4.4,-0.3)	$2.7x10^{-2}$	-0.4 (-2.0,1.2)	0.63	$5.5x10^{-2}$
rs10486445	7p15.3	OSBPL3	2.8 (0.3,5.3)	$2.7x10^{-2}$	0.2 (-1.9,2.4)	0.85	$7.1x10^{-2}$	1.6 (-0.7,3.9)	0.16	-0.2 (-2.4,2.0)	0.85	0.30
rs12530817			3.2 0.5,6.0)	$2.3x10^{-2}$	0.1 (-2.3,2.5)	0.94	$6.7x10^{-2}$	2.1 (-0.5,4.6)	0.11	0.2 (-2.3,2.6)	0.88	0.23
rs4722856	7p14.3	CREB5	1.1 (-0.3,2.6)	0.13	0.5 (-0.8,1.7)	0.47	0.16	1.9 (0.5,3.2)	$7.6x10^{-3}$	2.5 (1.3,3.8)	$8.1x10^{-5}$	$5.1x10^{-6}$
rs7806653	7q34	ATP6V0A4	5.0 (0.9,9.2)	$1.8x10^{-2}$	0.8 (-2.1,3.8)	0.58	$3.6x10^{-2}$	2.0 (-1.5,5.6)	0.26	0.5 (-2.5,3.5)	0.74	0.39
rs12248820	10q23.1	SH2D4B	-1.7 (-2.9,-0.5)	$5.4x10^{-3}$	-0.9 (-2.0,0.1)	$8.1x10^{-2}$	$2.2x10^{-3}$	-1.8 (-2.9,-0.6)	$2.2 x10^{-3}$	-1.1 (-2.2,-0.1)	$3.2 x10^{-2}$	$2.5x10^{-4}$
rs2515376	11q22.1	CNTN5	1.8 (0.6,3.0)	$2.4x10^{-3}$	0.3 (-0.6,1.3)	0.52	$5.7x10^{-3}$	1.7 (0.7,2.7)	$1.3 x10^{-3}$	0.0 (-0.9,1.0)	0.94	$7.6x10^{-6}$
rs1795505	12q21.31	LIN7A	2.6 (0.8,4.3)	$3.7x10^{-3}$	0.3 (-1.1,1.7)	0.68	$1.1x10^{-2}$	2.0 (0.5,3.6)	$1.2 x10^{-2}$	1.2 (-0.2,2.6)	$9.5 x10^{-2}$	$5.2x10^{-3}$
rs1184776			2.6 (0.8,4.3)	$3.5x10^{-3}$	0.3 (-1.1,1.8)	0.65	$9.6x10^{-3}$	2.1 (0.5,3.7)	$1.2 x10^{-2}$	1.3 (-0.1,2.7)	$7.8 x10^{-2}$	$4.4x10^{-3}$
rs1163683			2.4 (0.6,4.2)	$8.1x10^{-3}$	0.3 (-1.1,1.8)	0.65	$2.0x10^{-2}$	2.0 (0.3,3.6)	$1.9 x10^{-2}$	1.3 (-0.1,2.7)	$7.8 x10^{-2}$	$6.6x10^{-3}$

	3-4yrs post-RT					4-5yrs post-RT				
	Discovery Cohort		Replication Cohort			Discovery Cohort		Replication Cohort		
dbSNPrsID	Beta (95%CI)	p-value	Beta (95%CI)	p-value	Combined p-value	Beta (95%CI)	p-value	Beta (95%CI)	p-value	Combined p-value
rs3818568	1.4 (0.1,2.8)	$3.4x10^{-2}$	0.2 (-1.0,1.4)	0.750	$7.7x10^{-2}$	1.9 (0.4,3.4)	$1.5x10^{-2}$	0.3 (-1.0,1.5)	0.66	$3.5x10^{-2}$
rs13401638	5.6 (2.3,8.8)	$8.0x10^{-4}$	3.2 (0.9,5.6)	$8.2x10^{-3}$	$4.7x10^{-5}$	6.3 (2.7,9.9)	$6.4x10^{-4}$	2.0 (-0.4,4.5)	0.11	$7.0x10^{-4}$
rs13404973	5.6 (2.3,8.8)	$8.0x10^{-4}$	2.9 (0.7,5.2)	$1.2x10^{-2}$	$6.7x10^{-5}$	6.3 (2.7,9.9)	$6.4x10^{-4}$	2.0 (-0.4,4.5)	0.11	$7.0x10^{-4}$
rs13405086	5.6 (2.3,8.8)	$8.0x10^{-4}$	2.9 (0.7,5.2)	$1.2x10^{-2}$	$6.7x10^{-5}$	6.3 (2.7,9.9)	$6.4x10^{-4}$	2.0 (-0.4,4.5)	0.11	$7.0x10^{-4}$
rs4953253	4.6 (1.4,7.7)	$4.6x10^{-3}$	2.6 (0.5,4.7)	$1.5x10^{-2}$	$4.0x10^{-4}$	6.1 (2.5,9.7)	$1.0x10^{-3}$	1.5 (-0.7,3.8)	0.19	$1.1x10^{-3}$
rs13035033	6.4 (3.6,9.3)	$1.4x10^{-5}$	1.3 (-1.0,3.6)	0.26	$2.7x10^{-5}$	6.0 (2.5,9.4)	$9.3x10^{-4}$	2.3 (0.0,4.6)	$5.2x10^{-2}$	$3.0x10^{-4}$
rs9866974	0.7 (-1.2,2.7)	0.46	0.4 (-1.4,2.3)	0.64	0.53	1.9 (-0.3,4.0)	$8.7x10^{-2}$	0.4 (-1.5,2.4)	0.67	0.15
rs6764017	4.0 (2.1,6.0)	$5.6x10^{-5}$	1.6 (0.0,3.3)	$5.5x10^{-2}$	$2.3x10^{-5}$	1.3 (-0.8,3.5)	0.22	2.4 (0.7,4.2)	$7.3x10^{-3}$	$7.1x10^{-3}$
rs865808	2.0 (1.0,3.0)	$7.6x10^{-5}$	0.9 (0.1,1.7)	$3.2x10^{-2}$	$1.9x10^{-5}$	1.5 (0.3,2.6)	$1.7x10^{-2}$	1.2 (0.4,2.0)	$4.6x10^{-3}$	$5.0x10^{-4}$
rs7676349	4.0 (1.2,6.7)	$5.2x10^{-3}$	1.6 (-0.5,3.7)	0.13	$3.3x10^{-2}$	3.2 (0.4,6.1)	$2.7x10^{-2}$	3.6 (1.6,5.7)	$7.3x10^{-4}$	$9.7x10^{-5}$
rs41385744	5.6 (2.4,8.8)	$7.9x10^{-4}$	3.0 (0.7,5.4)	$1.2x10^{-2}$	$6.6x10^{-5}$	4.4 (1.0,7.8)	$1.1x10^{-2}$	3.6 (1.1,6.1)	$5.8x10^{-3}$	$4.0x10^{-4}$
rs16883632	3.0 (1.2,4.8)	$1.0x10^{-3}$	1.7 (0.2,3.2)	$3.1x10^{-2}$	$2.0x10^{-4}$	2.4 (0.6,4.2)	$9.2x10^{-3}$	2.6 (0.8,4.4)	$5.2x10^{-3}$	$3.0x10^{-4}$
rs9390419	1.7 (0.3,3.0)	$1.8x10^{-2}$	1.1 (0.0,2.2)	$5.6x10^{-2}$	$4.7x10^{-3}$	1.2 (-0.3,2.7)	0.13	1.7 (0.5,2.9)	$6.6x10^{-3}$	$4.1x10^{-3}$
rs9403813	1.9 (0.5,3.2)	$8.9x10^{-3}$	1.1 (0.0,2.2)	$5.8x10^{-2}$	$2.6x10^{-3}$	1.2 (-0.3,2.7)	0.13	1.9 (0.7,3.2)	$2.5x10^{-3}$	$1.7x10^{-3}$
rs6463707	-2.8 (-4.8,-0.7)	$9.7x10^{-3}$	-1.3 (-2.8,0.2)	$8.9x10^{-2}$	$4.1x10^{-3}$	-4.0 (-6.4,-1.5)	$1.8x10^{-3}$	-1.7 (-3.2,-0.2)	$3.0x10^{-2}$	$3.0x10^{-4}$
rs10486445	4.0 (1.7,6.3)	$7.6x10^{-4}$	2.2 (0.1,4.3)	$3.8x10^{-2}$	$2.0x10^{-4}$	3.6 (0.8,6.3)	$1.1x10^{-2}$	2.6 (0.4,4.7)	$2.3x10^{-2}$	$1.3x10^{-3}$
rs12530817	4.5 (2.1,7.0)	$3.9x10^{-4}$	2.3 (-0.1,4.7)	$5.9x10^{-2}$	$2.0x10^{-4}$	4.6 (1.7,7.4)	$2.1x10^{-3}$	2.5 (-0.1,5.1)	$6.1x10^{-2}$	$7.0x10^{-4}$
rs4722856	2.3 (0.9,3.7)	$1.2x10^{-3}$	1.4 (0.2,2.6)	$1.9x10^{-2}$	$1.0x10^{-4}$	1.8 (0.2,3.4)	$2.5x10^{-2}$	0.6 (-0.7,1.9)	0.36	$3.2x10^{-2}$
rs7806653	7.3 (3.3,11.3)	$3.6x10^{-4}$	3.5 (0.7,6.3)	$1.6x10^{-2}$	$4.2x10^{-5}$	6.6 (2.2,11.0)	$3.4x10^{-3}$	2.2 (-1.0,5.4)	0.19	$3.1x10^{-2}$
rs12248820	-1.9 (-3.0,-0.7)	$1.2x10^{-3}$	-0.6 (-1.6,0.3)	0.20	$1.3x10^{-3}$	-0.9 (-2.2,0.4)	0.17	-0.7 (-1.6,0.3)	0.19	$9.4x10^{-2}$
rs2515376	1.5 (0.4,2.5)	$7.3 x10^{-3}$	0.7 (-0.2,1.6)	0.12	$4.1x10^{-3}$	1.8 (0.6,3.0)	$3.3 x10^{-3}$	1.1 (0.2,2.0)	$1.6x10^{-2}$	$3.0x10^{-4}$
rs1795505	2.3 (0.6,3.9)	$6.5 x10^{-3}$	1.5 (0.1,2.8)	$3.2 x10^{-2}$	$1.1x10^{-3}$	1.5 (-0.3,3.3)	0.11	0.6 (-0.8,2.0)	0.40	0.12
rs1184776	2.4 (0.7,4.0)	$4.4 x10^{-3}$	1.5 (0.2,2.9)	$2.6 x10^{-2}$	$7.0x10^{-4}$	1.7 (-0.1,3.5)	$7.1 x10^{-2}$	0.6 (-0.8,2.0)	0.40	$8.5x10^{-2}$
rs1163683	2.3 (0.7,3.9)	$5.9 x10^{-3}$	1.5 (0.2,2.9)	$2.6 x10^{-2}$	$9.0x10^{-4}$	1.6 (-0.2,3.4)	$8.3 x10^{-2}$	0.6 (-0.8,2.0)	0.40	$9.6x10^{-2}$

Figure 3. Genomic structure of the 9p22.1 locus harboring the 8 SNPs that form a haplotype associated with change in AUASS following RT. SNPs identified in this study are marked with *. Numbers denote linkage disequilibrium r^2 values for pairs of these SNPs.



	1-2yrs post-RT			2-3yrs post-RT			3-4yrs post-RT			4-5yrs post-RT		
	Disc. Beta (95%CI)	Rep. Beta (95%CI)	Combined p-value	Disc. Beta (95%CI)	Rep. Beta (95%CI)	Combined p-value	Disc. Beta (95%CI)	Rep. Beta (95%CI)	Combined p-value	Disc. Beta (95%CI)	Rep. Beta (95%CI)	Combined p-value
CATAGGTG haplotype	1.4 (-0.2,3.0)	1.1 (-0.1,2.3)	$2.7x10^{-2}$	2.7 (1.2,4.12)	1.6 (0.4,2.8)	$2.6x10^{-5}$	2.9 (1.4,4.4)	1.2 (0.1,2.4)	$3.7x10^{-5}$	2.4 (0.7,4.01)	0.4 (-0.8,1.5)	$1.2x10^{-2}$

Table 3. A. Univariate linear regression of clinical and treatment variables on change in AUASS after RT. Change in AUASS was analyzed at four time periods following RT. B. Multivariate linear regression of clinical and treatment variables on change in AUASS after RT. *Age was scaled so that a one-unit increase is equal to 5 years; initial PSA was scaled so that a one-unit increase is equal to 5ng/ml; total BED was scaled to that a one-unit increase is equal to 10Gy2.

A

	1-2yrs		2-3yrs		3-4yrs		4-5yrs	
	Beta (95% CI)	**p-value**	**Beta (95% CI)**	**p-value**	**Beta (95% CI)**	**p-value**	**Beta (95% CI)**	**p-value**
Age (years)*	-0.4 (-0.8,-0.1)	**0.024**	-0.1 (-0.5,0.2)	0.512	0.0 (-0.4,0.3)	0.878	0.0 (-0.4,0.4)	0.825
Stage								
T1	Ref.		Ref.		Ref.		Ref.	
T2	-0.1 (-1.2,1.0)	0.396	0.1 (-0.9,1.2)	0.118	0.2 (-0.9,1.2)	0.108	0.1 (-1.0,1.3)	0.971
T3	-2.0 (-4.9,0.9)		-2.8 (-5.6,0.0)		-2.9 (-5.7,-0.1)		-0.1 (-3.3,3.2)	
Gleason score								
≤ 6	Ref.		Ref.		Ref.		Ref.	
7	-0.2 (-1.4,1.0)	0.381	-0.6 (-1.8,0.6)	0.081	0.0 (-1.2,1.2)	0.565	0.5 (-0.8,1.8)	0.655
≥ 8	-1.2 (-2.9,0.5)		-1.8 (-3.5,-0.2)		-0.9 (-2.6,0.7)		-0.3 (-2.3,1.6)	
TURP prior to RT	-2.4 (-5.6,0.7)	0.133	-1.2 (-4.2,1.8)	0.429	-0.1 (-3.0,2.7)	0.918	-0.5 (-3.8,2.7)	0.737
Initial PSA (ng/ml)*	-0.1 (-0.2,0.1)	0.495	-0.1 (-0.3,0.0)	0.113	-0.1 (-0.3,0.1)	0.262	-0.1 (-0.3,0.1)	0.331
Androgen deprivation therapy	-1.4 (-2.4,-0.3)	**0.012**	-1.1 (-2.1,-0.1)	**0.040**	-0.9 (-1.9,0.1)	0.089	-0.5 (-1.7,0.6)	0.347
Treatment								
Brachytherapy only	Ref.	0.664	Ref.	0.566	Ref.	0.927	Ref.	0.253
Brachytherapy + EBRT	0.2 (-0.8,1.3)		-0.3 (-1.3,0.7)		-0.1 (-1.1,1.0)		0.7 (-0.5,1.8)	
Total BED (Gy2)*	0.1 (-0.1,0.4)	0.222	-0.1 (-0.3,0.1)	0.369	-0.1 (-0.3,0.1)	0.320	0.1 (-0.2,0.3)	0.682
Prostate volume (mm^2)	0.1 (-0.1,0.2)	0.465	0.0 (-0.1,0.2)	0.760	0.0 (-0.2,0.1)	0.857	0.0 (-0.2,0.1)	0.685
Smoker	1.1 (0.0,2.2)	**0.045**	1.0 (0.0,2.1)	**0.048**	0.4 (-0.6,1.5)	0.399	1.1 (0.0,2.3)	0.050
Hypertension	1.3 (0.1,2.4)	**0.026**	0.8 (-0.3,1.8)	0.176	1.0 (-0.1,2.1)	0.069	1.2 (0.0,2.4)	0.058
Diabetes	0.2 (-2.0,2.5)	0.849	1.6 (-0.7,3.8)	0.165	0.7 (-1.6,2.9)	0.560	0.3 (-2.1,2.7)	0.827
Use of alpha-blocker following RT	1.5 (0.4,2.5)	**0.006**	0.6 (-0.4,1.7)	0.220	0.3 (-0.7,1.3)	0.587	0.0 (-1.2,1.1)	0.956
Pre-RT AUASS								
0-7	Ref.		Ref.		Ref.		Ref.	
8-19	-3.1 (-4.1,-2.0)	**<0.001**	-3.5 (-4.6,-2.5)	**<0.001**	-3.8 (-4.8,-2.8)	**<0.001**	-3.9 (-5.0,-2.7)	**<0.001**
20-35	-7.5 (-9.8,-5.2)		-8.2 (-10.5,-6.0)		-7.4 (-9.6,-5.1)		-8.9 (-11.3,-6.5)	

B

	1-2yrs		2-3yrs		3-4yrs		4-5yrs	
	Beta (95% CI)	**p-value**	**Beta (95% CI)**	**p-value**	**Beta (95% CI)**	**p-value**	**Beta (95% CI)**	**p-value**
Age (years)*	-0.3 (-0.7,0.0)	0.056	0.0 (-0.4,0.3)	0.843	0.0 (-0.3,0.4)	0.802	0.0 (-0.3,0.4)	0.886
Androgen deprivation therapy	-0.9 (-1.9,0.2)	0.100	-0.7 (-1.7,0.3)	0.184	-0.5 (-1.5,0.5)	0.322	0.0 (-1.1,1.1)	0.957
Smoker	1.1 (0.1,2.1)	**0.041**	1.2 (0.3,2.2)	**0.012**	0.5 (-0.5,1,4)	0.356	1.1 (0.1,2.2)	**0.040**
Hypertension	1,1 (0.1,2.2)	**0.039**	0.5 (-0.6,1.5)	0.363	0.8 (-0.2,1.8)	0.128	0.9 (-0.2,2.1)	0.118
Use of alpha-blockers following RT	2.3 (1.2,3.3)	**<0.001**	1.5 (0.5,2.5)	**0.003**	1.1 (0.1,2.10	**0.028**	0.7 (-0.4,1.7)	0.237
Pre-RT AUASS								
0-7	Ref.		Ref.		Ref.		Ref.	
8-19	-3.4 (-4.5,-2.3)	**<0.001**	-3.9 (-4.9,-2,8)	**<0.001**	-4.0 (-5.1,-3.0)	**<0.001**	-4.1 (-5.3,-2.9)	**<0.001**
20-35	-7.8 (-10.2,-5.5)		-8.5 (-10.8,-6.3)		-7.5 (-9.8,-5.2)		-8.8 (-11.2,-6.4)	

Supplementary Table 2. Association between SNPs on 9p21.2 and change in each AUASS question during the follow-up period identified previously has showing the strongest association between each SNP and change in total AUASS, as indicated. Results are from linear regression among all patients (N=723) adjusted for hypertension at diagnosis, alpha-blocker use, smoking history, pre-RT score for the given AUASS item and PCs1-5. SNPs showing selective association with one or more AUASS items in particular are shaded.

		Incomplete emptying		Frequency		Intermittency		Urgency		Weak stream		Straining		Nocturia	
SNP	Follow-up period	Beta (95% CI)	p-value	Beta (95% CI)	p-value	Beta (95% CI)	p-value	Beta (95% CI)	p-value	Beta (95% CI)	p-value	Beta (95% CI)	p-value	Beta (95% CI)	p-value
rs3818568	1-2yrs	0.3 (0.2,0.5)	$2.1x10^{-4}$	0.3 (0.1,0.4)	$9.6x10^{-3}$	0.3 (0.1,0.5)	$2.1x10^{-3}$	0.3 (0.1,0.5)	$1.0x10^{-2}$	0.2 (0.0,0.4)	$4.5x10^{-2}$	0.1 (0.0,0.3)	$5.4x10^{-2}$	0.1 (-0.1,0.2)	0.23
rs13401638	3-4yrs	0.8 (0.4,1.2)	$2.3x10^{-5}$	0.6 (0.1,1.0)	$1.1x10^{-2}$	0.7 (0.3,1.0)	$9.8x10^{-4}$	0.7 (0.3,1.1)	$7.8x10^{-4}$	0.8 (0.3,1.2)	$5.6x10^{-4}$	0.5 (0.2,0.8)	$1.3x10^{-3}$	0.3 (-0.1,0.7)	0.11
rs13404973	3-4yrs	0.8 (0.4,1.1)	$3.3x10^{-5}$	0.5 (0.1,1.0)	$1.1x10^{-2}$	0.6 (0.2,1.0)	$1.4x10^{-3}$	0.6 (0.2,1.1)	$2.3x10^{-3}$	0.8 (0.3,1.2)	$6.0x10^{-4}$	0.5 (0.2,0.8)	$7.4x10^{-4}$	0.3 (-0.1,0.6)	0.12
rs13405086	3-4yrs	0.8 (0.4,1.1)	$3.3x10^{-5}$	0.5 (0.1,1.0)	$1.1x10^{-2}$	0.6 (0.2,1.0)	$1.4x10^{-3}$	0.6 (0.2,1.1)	$2.3x10^{-3}$	0.8 (0.3,1.2)	$6.0x10^{-4}$	0.5 (0.2,0.8)	$7.3x10^{-4}$	0.3 (-0.1,0.6)	0.12
rs4953253	3-4yrs	0.7 (0.3,1.1)	$1.6x10^{-4}$	0.5 (0.1,0.9)	$1.4x10^{-2}$	0.5 (0.1,0.8)	$7.6x10^{-3}$	0.7 (0.4,1.1)	$2.0x10^{-4}$	0.6 (0.2,1.0)	$4.6x10^{-3}$	0.4 (0.1,0.6)	$1.3x10^{-2}$	0.2 (-0.1,0.5)	0.22
rs13035033	2-3yrs	0.4 (0.0,0.8)	$5.1x10^{-2}$	0.4 (0.0,0.8)	$8.5x10^{-2}$	0.3 (-0.1,0.7)	0.12	0.6 (0.2,1.1)	$5.9x10^{-3}$	0.6 (0.1,1.0)	$1.1x10^{-2}$	0.9 (0.6,1.2)	$5.0x10^{-9}$	0.3 (-0.1,0.6)	0.14
rs9866974	2-3yrs	0.2 (-0.1,0.5)	0.14	0.4 (0.2,0.7)	$1.5x10^{-3}$	0.3 (0.0,0.5)	$4.1x10^{-2}$	0.3 (0.0,0.6)	$7.0x10^{-2}$	0.6 (0.3,0.9)	$9.9x10^{-5}$	0.3 (0.1,0.5)	$1.8x10^{-3}$	0.4 (0.2,0.6)	$4.6x10^{-4}$
rs6764017	3-4yrs	0.3 (0.0,0.5)	$3.8x10^{-2}$	0.4 (0.1,0.6)	$1.5x10^{-2}$	0.1 (-0.1,0.4)	0.29	0.4 (0.1,0.7)	$2.8x10^{-3}$	0.2 (-0.1,0.5)	0.26	0.2 (0.0,0.4)	0.11	0.4 (0.1,0.6)	$1.4x10^{-3}$
rs865808	3-4yrs	0.2 (0.1,0.3)	$5.7x10^{-3}$	0.2 (0.1,0.4)	$3.4x10^{-4}$	0.2 (0.1,0.3)	$1.3x10^{-3}$	0.2 (0.1,0.4)	$9.8x10^{-4}$	0.2 (0.1,0.4)	$1.4x10^{-3}$	0.2 (0.1,0.3)	$8.3x10^{-4}$	0.2 (0.1,0.3)	$3.5x10^{-3}$
rs7676349	4-5yrs	0.5 (0.1,0.8)	$7.8x10^{-3}$	0.4 (0.0,0.8)	$3.2x10^{-2}$	0.6 (0.2,0.9)	$1.4x10^{-3}$	0.5 (0.2,0.9)	$4.6x10^{-3}$	0.8 (0.4,1.1)	$4.6x10^{-5}$	0.4 (0.1,0.6)	$7.3x10^{-3}$	0.3 (-0.1,0.6)	0.11
rs41385744	4-5yrs	0.7 (0.3,1.1)	$1.3x10^{-3}$	0.6 (0.2,1.1)	$4.5x10^{-3}$	0.5 (0.0,0.9)	$3.4x10^{-2}$	0.5 (0.1,1.0)	$2.3x10^{-2}$	0.7 (0.3,1.2)	$1.2x10^{-3}$	0.4 (0.0,0.7)	$2.6x10^{-2}$	0.3 (-0.1,0.7)	0.12
rs16883632	3-4yrs	0.3 (0.1,0.5)	$1.7x10^{-2}$	0.4 (0.2,0.7)	$1.2x10^{-3}$	0.4 (0.2,0.7)	$2.8x10^{-4}$	0.3 (0.1,0.6)	$8.3x10^{-3}$	0.4 (0.1,0.7)	$3.8x10^{-3}$	0.3 (0.1,0.5)	$3.6x10^{-3}$	0.2 (0.0,0.4)	0.12
rs9390419	2-3yrs	0.3 (0.1,0.5)	$1.1x10^{-3}$	0.2 (0.0,0.4)	$2.6x10^{-2}$	0.2 (0.0,0.4)	$2.2x10^{-2}$	0.3 (0.1,0.5)	$2.9x10^{-3}$	0.3 (0.1,0.5)	$7.3x10^{-4}$	0.3 (0.2,0.5)	$2.7x10^{-6}$	0.1 (-0.1,0.2)	0.32
rs9403813	2-3yrs	0.3 (0.1,0.4)	$3.4x10^{-3}$	0.2 (0.0,0.4)	$2.8x10^{-2}$	0.2 (0.0,0.3)	$5.2x10^{-2}$	0.3 (0.1,0.5)	$7.7x10^{-3}$	0.3 (0.1,0.5)	$1.3x10^{-3}$	0.3 (0.2,0.5)	$2.4x10^{-6}$	0.1 (-0.1,0.2)	0.37
rs6463707	4-5yrs	-0.4 (-0.6,-0.1)	$5.7x10^{-3}$	-0.3 (-0.6,0.0)	$4.7x10^{-2}$	-0.4 (-0.7,-0.1)	$3.2x10^{-3}$	-0.4 (-0.7,-0.1)	$3.0x10^{-3}$	-0.4 (-0.7,-0.1)	$1.3x10^{-2}$	-0.2 (-0.5,0.0)	$2.5x10^{-2}$	-0.1 (-0.3,0.2)	0.54
rs10486445	3-4yrs	0.5 (0.2,0.8)	$8.6x10^{-4}$	0.4 (0.1,0.8)	$1.0x10^{-2}$	0.4 (0.0,0.7)	$2.5x10^{-2}$	0.7 (0.4,1.0)	$3.8x10^{-5}$	0.4 (0.1,0.8)	$1.4x10^{-2}$	0.4 (0.1,0.6)	$1.9x10^{-3}$	0.5 (0.2,0.8)	$3.1x10^{-4}$
rs12530817	3-4yrs	0.5 (0.2,0.8)	$3.2x10^{-3}$	0.6 (0.2,1.0)	$1.8x10^{-3}$	0.4 (0.1,0.8)	$1.9x10^{-2}$	0.9 (0.5,1.3)	$1.3x10^{-6}$	0.6 (0.2,0.9)	$4.8x10^{-3}$	0.4 (0.1,0.7)	$3.1x10^{-3}$	0.6 (0.3,0.9)	$3.2x10^{-4}$
rs4722856	2-3yrs	0.2 (0.0,0.4)	$3.6x10^{-2}$	0.2 (0.0,0.4)	$1.5x10^{-2}$	0.4 (0.2,0.6)	$0.2x10^{-6}$	0.2 (-0.1,0.4)	0.15	0.3 (0.1,0.5)	$2.8x10^{-3}$	0.2 (0.0,0.3)	$1.1x10^{-2}$	0.3 (0.1,0.4)	$1.1x10^{-3}$
rs7806653	3-4yrs	0.7 (0.3,1.2)	$2.4x10^{-3}$	0.7 (0.2,1.2)	$2.0x10^{-2}$	0.6 (0.1,1.1)	$1.5x10^{-2}$	0.9 (0.4,1.4)	$6.2x10^{-4}$	0.8 (0.2,1.3)	$4.7x10^{-3}$	0.8 (0.5,1.2)	$7.7x10^{-6}$	0.4 (-0.1,0.8)	$8.4x10^{-2}$
rs17779457	2-3yrs	0.4 (0.2,0.6)	$2.1x10^{-5}$	0.5 (0.3,0.7)	$1.3x10^{-6}$	0.4 (0.3,0.6)	$4.5x10^{-6}$	0.3 (0.1,0.5)	$7.9x10^{-3}$	0.2 (0.0,0.4)	$4.1x10^{-2}$	0.1 (-0.1,0.2)	0.49	0.2 (0.0,0.3)	$4.2x10^{-2}$
rs10812604	2-3yrs	0.4 (0.2,0.5)	$4.7x10^{-4}$	0.4 (0.2,0.6)	$1.2x10^{-5}$	0.4 (0.2,0.6)	$2.5x10^{-5}$	0.3 (0.1,0.5)	$8.9x10^{-3}$	0.2 (0.0,0.5)	$2.7x10^{-2}$	0.1 (-0.1,0.3)	0.25	0.2 (0.1,0.4)	$6.3x10^{-3}$
rs10967965	2-3yrs	0.5 (0.3,0.7)	$6.5x10^{-6}$	0.5 (0.3,0.7)	$9.4x10^{-6}$	0.4 (0.2,0.6)	$6.2x10^{-5}$	0.3 (0.1,0.6)	$4.7x10^{-3}$	0.4 (0.1,0.6)	$2.3x10^{-3}$	0.2 (0.0,0.4)	$9.1x10^{-2}$	0.2 (0.0,0.4)	$2.0x10^{-2}$
rs1537712	2-3yrs	0.3 (0.1,0.5)	$7.3x10^{-4}$	0.5 (0.3,0.7)	$8.5x10^{-7}$	0.4 (0.2,0.6)	$5.3x10^{-5}$	0.4 (0.1,0.6)	$1.3x10^{-3}$	0.2 (0.0,0.5)	$2.0x10^{-2}$	0.1 (-0.1,0.2)	0.40	0.1 (0.0,0.3)	0.11
rs774354	2-3yrs	0.3 (0.1,0.5)	$6.7x10^{-4}$	0.5 (0.3,0.7)	$1.1x10^{-6}$	0.4 (0.2,0.6)	$3.5x10^{-5}$	0.4 (0.1,0.6)	$1.3x10^{-3}$	0.2 (0.0,0.5)	$2.4x10^{-2}$	0.1 (-0.1,0.2)	0.39	0.1 (0.0,0.3)	$8.4x10^{-2}$
rs774352	2-3yrs	0.3 (0.1,0.5)	$7.4x10^{-4}$	0.5 (0.3,0.7)	$4.6x10^{-7}$	0.4 (0.2,0.6)	$4.3x10^{-5}$	0.4 (0.1,0.6)	$1.1x10^{-3}$	0.2 (0.0,0.4)	$3.5x10^{-2}$	0.1 (-0.1,0.2)	0.44	0.1 (0.0,0.3)	0.10
rs700782	2-3yrs	0.4 (0.2,0.6)	$1.1x10^{-4}$	0.5 (0.3,0.7)	$1.5x10^{-6}$	0.4 (0.2,0.6)	$5.7x10^{-6}$	0.3 (0.1,0.5)	$4.9x10^{-3}$	0.2 (0.0,0.4)	$2.9x10^{-2}$	0.1 (-0.1,0.2)	0.46	0.2 (0.0,0.4)	$1.9x10^{-2}$
rs2453552	2-3yrs	0.4 (0.2,0.6)	$6.8x10^{-5}$	0.4 (0.2,0.6)	$1.0x10^{-5}$	0.4 (0.2,0.6)	$4.8x10^{-5}$	0.3 (0.1,0.5)	$4.6x10^{-3}$	0.2 (0.0,0.4)	$3.6x10^{-2}$	0.0 (-0.1,0.2)	0.73	0.2 (0.0,0.4)	$3.2x10^{-2}$
rs12248820	2-3yrs	0.1 (-0.1,0.2)	0.25	0.2 (0.0,0.3)	$5.2x10^{-2}$	0.2 (0.0,0.3)	$2.5x10^{-2}$	0.1 (-0.1,0.3)	0.31	0.2 (0.1,0.4)	$5.8x10^{-3}$	0.2 (0.1,0.3)	$2.4x10^{-4}$	0.0 (-0.1,0.2)	0.59
rs2515376	4-5yrs	0.3 (0.1,0.4)	$4.3x10^{-4}$	0.2 (0.0,0.3)	$5.0x102^{-}$	0.2 (0.0,0.3)	$2.9x10^{-2}$	0.3 (0.1,0.4)	$1.3x10^{-3}$	0.3 (0.1,0.5)	$3.0x10^{-4}$	0.2 (0.1,0.3)	$3.5x10^{-3}$	0.1 (0.0,0.3)	0.14
rs1795505	3-4yrs	0.4 (0.2,0.6)	$1.4x10^{-4}$	0.3 (0.1,0.5)	$1.4x10^{-2}$	0.4 (0.2,0.6)	$3.9x10^{-4}$	0.3 (0.0,0.5)	$1.9x10^{-2}$	0.4 (0.1,0.6)	$2.0x10^{-3}$	0.1 -(0.1,0.3)	0.20	0.2 (0.0,0.4)	$4.5x10^{-2}$
rs1184776	3-4yrs	0.4 (0.2,0.6)	$6.2x10^{-5}$	0.3 (0.1,0.5)	$1.2x10^{-2}$	0.4 (0.2,0.6)	$4.4x10^{-4}$	0.3 (0.1,0.5)	$1.2x10^{-2}$	0.4 (0.2,0.6)	$1.1x10^{-3}$	0.1 (0.0,0.3)	0.15	0.2 (0.0,0.4)	$3.5x10^{-2}$
rs1163683	3-4yrs	0.4 (0.2,0.6)	$1.1x10^{-4}$	0.3 (0.1,0.5)	$1.3x10^{-2}$	0.4 (0.2,0.6)	$3.5x10^{-4}$	0.3 (0.1,0.5)	$8.1x10^{-3}$	0.4 (0.2,0.6)	$1.1x10^{-3}$	0.1 (0.0,0.3)	0.11	0.2 (0.0,0.4)	$5.8x10^{-2}$